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BY
MATIAS NILSSON

DISSERTATION SUBMITTED 2015

AALBORG UNIVERSITY
DENMARK

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- **Nilsson M**, Sandberg TH, Poulsen JL, Gram M, Frøkjær JB, Østergaard LR, Krogh K, Brock C & Drewes AM. Quantification and Variability of Colonic Volume with a Novel Magnetic Resonance Imaging Method. *J Neurogastroenterol Motil* (2015).
- Sandberg TH, **Nilsson M**, Poulsen JL, Gram M, Frøkjær JB, Østergaard LR & Drewes AM. A novel semi-automatic segmentation method for volumetric assessment of the colon based on magnetic resonance imaging. *Abdom Imaging* (2015).
- Botha C, Farmer AD, **Nilsson M**, Brock C, Drewes AM, Knowles CH & Aziz Q. Preliminary report: modulation of parasympathetic nervous system tone influences oesophageal pain hypersensitivity. *Gut* 64:611-6117 (2014).
- **Nilsson M**, Lassen D, Andresen T, Nielsen AK, Arendt-Nielsen L & Drewes AM. Intradermal glutamate and capsaicin injections: Intra- and inter-individual variability of provoked hyperalgesia and allodynia. *Clin Exp Pharmacol Physiol* 41(6): 423-429 (2014).
- **Nilsson M**, Piasco A, Nissen TD, Graversen C, Gazerani P, Lucas MF, Dahan A, Drewes AM & Brock C. Reproducibility of psychophysics and electroencephalography during offset analgesia. *Eur J Pain* 18(6): 824-834 (2013).

LIST OF PAPERS

This thesis was based on the following papers:

I. Nilsson M, Sandberg TH, Poulsen JL, Gram M, Frøkjær JB, Østergaard LR, Krogh K, Brock C, Drewes AM. Quantification and Variability of Colonic Volume with a Novel Magnetic Resonance Imaging Method. Neurogastroenterol Motil 2015 (in press)

II. Nilsson M, Poulsen JL, Brock C, Sandberg TH, Gram M, Frøkjær JB, Krogh K, Drewes AM. Opioid-induced Bowel Dysfunction in Healthy Volunteers Assessed with Questionnaires and Magnetic Resonance Imaging. Submitted: Eur J Gastroenterol Hepatol 2015.

III. Nilsson M, Brock C, Poulsen JL, Bindslev N, Hansen MB, Christrup LL, Drewes AM. Short-Term Oxycodone Treatment does not Affect Electrogenic Ion Transport in Isolated Mucosa from the Human Rectosigmoid Colon. Submitted: Scand J Gastroenterol 2015

IV. Poulsen JL, Nilsson M, Brock C, Sandberg TH, Krogh K, Drewes AM. The Impact of Opioid Treatment on Regional Gastrointestinal Transit. Submitted: J Neurogastroenterol Motil 2015.

ABBREVIATIONS

BFI:	Bowel function index
BSFS	Bristol stool form scale
cAMP:	Cyclic adenosine monophosphate
CFTR:	Cystic fibrosis conductance regulator
ClC-2	Chloride channel type-2
CNS:	Central nervous system
DOR:	δ-opioid receptor
ENS:	Enteric nervous system
FLIP:	Functional lumen imaging probe
GI:	Gastrointestinal
GSRS:	Gastrointestinal symptom rating scale
HV:	Healthy volunteer
KOR:	κ-opioid receptor
MOR:	μ-opioid receptor
MRI:	Magnetic resonance imaging
OIBD:	Opioid-induced bowel dysfunction
PAC-SYM:	Patient assessment of constipation symptom questionnaire
PGE_2:	Prostaglandin E_2
SOWS:	Subjective opiate withdrawal scale
STAI:	Spielberger's state-trait anxiety inventory
VAS:	Visual analogue scale

ENGLISH SUMMARY

The analgesic effect of opioids has been thoroughly investigated and has been known for millennia. Today opioids are used to treat both acute and chronic pain disorders. Strong opioids include morphine, methadone, fentanyl, oxycodone, and buprenorphine whereas codeine and tramadol are considered weak opioids. Unfortunately, the attractive analgesic properties of opioids are compromised by numerous gastrointestinal adverse effects, collectively known as opioid-induced bowel dysfunction (OIBD). One of the most prevalent adverse effects is constipation, which can be debilitating for the patients. Accordingly, constipation has been described thoroughly in the past. The treatment of OIBD is based on conventional alleviation of constipation, which does not account for the underlying pathophysiology. More detailed knowledge about the underlying pathophysiological mechanisms of opioid treatment on the gastrointestinal tract would therefore be beneficial to enhance our understanding of OIBD. Ultimately, the goal is to improve pain management through a reduction of adverse effects.

The objective of this Ph.D. dissertation was to investigate these adverse effects in healthy volunteers with a novel approach based on both subjective and objective methods. The methods include 1) several validated questionnaires on gastrointestinal function, 2) assessment of segmental colorectal volumes using magnetic resonance imaging (MRI), 3) assessment of gut secretion with Ussing chambers, 4) assessment of gastrointestinal transit times with a novel ambulatory capsule system (3D-Transit), and 5) assessment of anal sphincter function and distensibility the functional lumen imaging probe (FLIP). The hypothesis was that a more nuanced and complete picture of OIBD as an entity could be obtained through the combination of these subjective and objective measures.

Data was acquired from one randomised controlled trial where 25 healthy volunteers were treated with oxycodone or placebo in a double-blinded crossover design. The main results first and foremost included the successful development of a well-tolerated model of OIBD in healthy volunteers, based on questionnaire scores. During oxycodone treatment all subjects experienced a substantial impact on gut function with development of numerous OIBD symptoms (constipation, abdominal pain, bloating, straining, etc.) compared to placebo treatment. In paper I we compared MRI-based assessments of segmental colorectal volumes from the two baselines (before oxycodone and placebo treatment) to investigate whether the method was reproducible. The method showed low variability and was sensitive to assess the changes in segmental colorectal volumes that occur from defecation. Hence, the method was suitable for assessing changes in segmental colorectal volumes brought on by opioid treatment, which was investigated in paper II. Here we found that the volume for the caecum/ascending colon increased significantly during oxycodone treatment compared to placebo. In paper III we investigated whether opioid treatment alters gut secretion, using the Ussing chamber technique. We found that, at least for isolated mucosa from the rectosigmoid colon, no change in gut secretion occurred from opioid treatment. In paper IV gastrointestinal transit times were significantly prolonged during oxycodone treatment compared to placebo in the caecum/ascending colon and rectosigmoid colon. In the caecum/ascending colon the prolonged transit time induced by oxycodone

could be the underlying explanation behind the observation from paper II that the volume increased in this segment as well. Lastly, preliminary analysis of anal sphincter function and distensibility exhibited a large degree of variability, which may have obscured any oxycodone-induced alterations and more advanced analysis of this data is pending.

In conclusion, we have successfully developed an experimental model of OIBD in a controlled environment. The combination of the applied subjective and objective assessments enables a more thorough examination of the clinical manifestation and underlying pathophysiology of OIBD as a whole.

DANSK RESUME

Opioidernes smertelindrende effekt er særdeles velbeskrevet og har været kendt i tusinder af år. Således bliver de i dag anvendt til både akutte og kroniske svært behandlelige smerter. Stærke opioider dækker blandt andre over morfin, metadon, fentantyl, oxycodon og buprenorphin, hvorimod lægemidler som tramadol og kodein regnes som svage opioider. Desværre bliver effektiv smertelindring ofte kompromitteret af en række gastrointestinale bivirkninger, der under én fælles betegnelse kaldes opioid-induceret mavetarm-dysfunktion ('opioid-induced bowel dysfunction': OIBD). Heriblandt er forstoppelse en af de mest hyppigt forekommende bivirkninger, til stor gene for patienten og derfor desuden den hidtil bedst beskrevne bivirkning. Behandling af OIBD tager udgangspunkt i konventionel behandling af forstoppelse, hvilket ikke tager højde for den underliggende patofysiologi. Mere detaljeret viden om de underliggende patofysiologiske mekanismer på mavetarm-kanalen vil derfor være gavnligt. En øget forståelse for OIBD vil kunne bidrage til at reducere bivirkningerne, hvilket i sidste ende vil kunne optimere effektiv smertebehandling for den individuelle patient.

Målsætningen for denne ph.d.-afhandling er, at undersøge disse bivirkninger hos raske frivillige med en ny tilgang baseret på både subjektive og objektive metoder. Metoderne omfatter 1) flere validerede spørgeskemaer omhandlende gastrointestinal funktion, 2) måling af segmentale kolorektale volumina ved hjælp af MR-scanninger, 3) måling af tarmens sekretoriske respons med Ussingkamre, 4) vurdering af gastrointestinale transittider ved brug af et nyt ambulant kapsel-system (3D-Transit) samt 5) måling af den anale sfinkters funktion og distensibilitet med 'functional lumen imaging probe' (FLIP). Hypotesen var, at gennem kombinationen af disse subjektive og objektive metoder, ville et mere nuanceret og komplet billede af OIBD som helhed kunne opnås.

Data blev optaget fra ét randomiseret kontrolleret forsøg, hvor 25 raske frivillige blev behandlet med oxycodon eller placebo i et dobbelt-blindet overkrydsningsdesign. De vigtigste resultater omfatter først og fremmest den succesfulde etablering af en veltolereret model af OIBD i raske frivillige, baseret på spørgeskema-evalueringer. Alle forsøgspersoner oplevede en væsentlig påvirkning af deres mavetarm-funktion med talrige OIBD-symptomer (forstoppelse, mavesmerter, oppustethed, etc.) under oxycodonbehandling sammenlignet med placebobehandling. I artikel I sammenlignedes magnetisk resonans (MR)-baserede målinger af kolorektale volumina før påbegyndelse af hhv. oxycodonbehandling og placebobehandling. Dette blev gjort med henblik på at undersøge, om metoden var reproducerbar. Metoden udviste lav variabilitet og var sågar i stand til at måle de ændringer, der forekom som følge af toiletbesøg. Derfor var metoden velegnet til at vurdere ændringer i segmentale kolorektale volumina, der fremkom som følge af opioidbehandling, hvilket blev undersøgt i artikel II. Her blev fastslået at volumen af coecum/colon ascendens steg betydeligt efter oxycodonbehandling sammenlignet med placebobehandling. I artikel III blev Ussingkammer-teknikken anvendt til at undersøge om opioidbehandling ændrede tarmsekretion. Det blev fundet, at oxycodonbehandling ikke påvirkede tarmsekretionen i isoleret mucosa fra rektosigmoideum. I artikel IV blev de gastrointestinale transittider undersøgt, hvor vi fandt en væsentligt forlænget transittid

under oxycodonbehandling sammenlignet med placebobehandling i coecum/colon ascendens og rektosigmoideum. I coecum/colon ascendens kunne den forlængede transittid være den underliggende forklaring på observationen fra artikel II, at volumen tillige blev øget i dette segment som følge af oxycodonbehandling. Slutteligt viste en præliminær dataanalyse af den anale sfinkters funktion og distensibilitet stor variabilitet i datasættet, hvilket kan have udvisket en potentiel effekt af oxycodonbehandling og en mere avanceret dataanalyse er undervejs.

Afslutningsvis konkluderes, at det er lykkedes at udvikle en eksperimentel model af OIBD i et kontrolleret miljø. Kombinationen af subjektive og objektive mål muliggør en mere dybdegående undersøgelse af den kliniske manifestation og underliggende patofysiologiske mekanismer af OIBD som helhed.

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Thanks to my family and friends for your support and indulgence, I realize my area of research does not always make for the most appropriate dinner conversation.

Den sidste tak er til Kirsten Wenneberg Pedersen, det mest positive og hjertevarme menneske jeg nogensinde har mødt. Dig glemmer jeg aldrig!

Matias Nilsson, October 2015, Vejle

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Chapter 1 Introduction

1.1 Chronic pain and opioid-induced bowel dysfunction

Pain is one of the most frequently presented symptoms in patients in the primary and secondary health care sector. While acute pain is a normal sensory experience that alerts the individual of actual or potential tissue damage, chronic pain is vastly different. Often defined as any pain lasting more than 12 weeks, chronic pain may originate from an initial injury or infection, or there may be a persistent inflammation or continuous tissue remodelling that leads to chronic pain, such as arthritis or diabetic neuropathy. Chronic pain is associated with decreased quality of life, and comprehensive socioeconomic consequences (Langley et al. 2010; Breivik 2012). In fact, the European prevalence of chronic pain ranges from 12% to 30% of adults (Breivik et al. 2006). In Denmark, the estimated number of people with chronic pain is approximately 20% (Sjøgren et al. 2009). Of the patients presenting with moderate to severe non-malignant chronic pain, opioids are often considered the best intervention to achieving adequate pain relief when treatment with paracetamol or non-steroidal anti-inflammatory drugs (NSAIDS) have proven inadequate (Pappagallo 2001). In Denmark, approximately 13% of patients with chronic pain receive opioids (Kurita et al. 2012) but studies have shown great variation across countries with up to 90% of patients with chronic pain receiving opioid treatment (Benyamin et al. 2008).

However, the analgesic effect achieved through opioid treatment comes at a cost: Adverse effects are common and regrettably often counterbalance the analgesic benefits. The analgesic effect is achieved through binding to specific opioid receptors within the central nervous system (CNS). Additionally, identical receptors are also expressed in the gastrointestinal (GI) tract, which are directly related to normal GI functions by way of the endogenous opioids (e.g. endorphins) (De Schepper et al. 2004). However, exogenous opioids in clinical doses saturate the endogenous opioid system in the GI tract and consequently disrupt normal GI function (Wood & Galligan 2004; Camilleri 2011; Holzer 2009). This GI interference manifests as a plethora of symptoms including gastro-oesophageal reflux, vomiting, bloating, abdominal pain, anorexia, hard and dry stools, constipation, and incomplete evacuation (**Figure 1**).

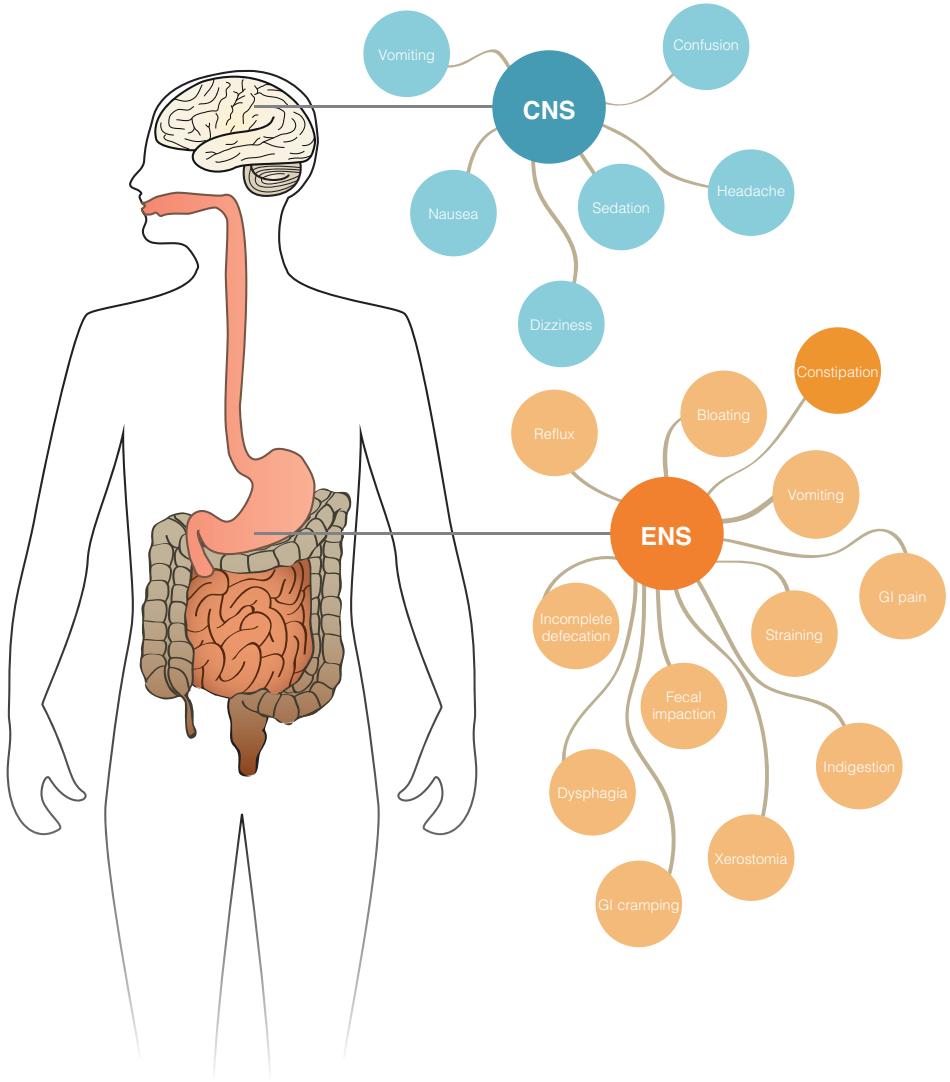

Figure 1: Opioid adverse effects on the central nervous system (CNS) and the enteric nervous system (ENS).

The GI symptoms related to adverse effects of exogenous opioids are collectively referred to under the umbrella-term 'opioid-induced bowel dysfunction' (OIBD) (Benyamin et al. 2008; Brock et al. 2012). These symptoms can be severe and drastically reduce patients' quality of life, which affects normal functioning and work productivity thereby carrying great socioeconomic impact (Bell et al. 2007; Cook et al. 2007; Penning-van Beest et al. 2010; Thorpe 2001). Adverse effects of opioid use also occur as a result of opioid binding within the CNS where common symptoms include nausea, sedation, headache, confusion, dizziness, and vomiting although tolerance to these adverse effects

tend to develop over time. In contrast, tolerance to OIBD is rarely achieved and because opioids inhibit GI function at doses much lower than those needed to produce analgesia these adverse effects cannot be easily overcome through opioid dose reductions (Swegle & Logemann 2006; Shook et al. 1987). In fact, opioid dosage tends to escalate over time and constipation becomes an increasing burden for the chronic pain patient (Pappagallo 2001). Cook et al. found that 57% of adults using opioids to manage non-cancer pain, reported developing constipation in association with opioid use (Cook et al. 2008). Development of constipation as a result of opioid treatment may lead to obstipation, colonic distension, ileus and even perforation (Dubinsky 1996). As a result, upwards of a third of patients treated with opioids report missing treatment, decreasing treatment, or even opt to completely discontinue treatment in order to improve bowel function. This impact on patient compliance naturally is a great obstacle in the attempt to provide adequate pain management as the resulting analgesic suboptimal treatment further impairs quality of life (Kurz & Sessler 2003; Bell et al. 2009; Cherny et al. 2001; Wirz 2005).

To overcome these adverse effects and increase patient compliance laxative co-administration is common in patients treated with opioids for chronic pain. Accordingly, Pappagallo et al., showed that 88% of 76 patients treated with opioids used at least one laxative and 58% of 76 patients used two or more different laxatives (Pappagallo 2001). The challenge with laxative use in the treatment of OIBD is that conventional laxatives (described in detail later) do not target the underlying pathophysiological mechanism, namely the binding of exogenous opioids to the opioid receptors in the enteric nervous system (ENS), and thus have limited effect on manifest OIBD.

1.1.1 The enteric nervous system (ENS)

Many aspects of OIBD have yet to be described and understood in detail. For example, it is not clear whether the bowel dysfunction is a pan-enteric phenomenon or if it primarily is the colon that is affected. Obviously, the lack of knowledge complicates effective alleviation of OIBD and the treatment options are mainly symptomatic and rely heavily on laxative use (although these often display relatively poor efficacy) for constipation caused by opioid use. The primary mechanism of action of conventional laxatives is mainly targeted at the colon, relying on increasing the osmotic gradient and/or stimulating the colonic musculature. Because OIBD likely affects peripheral opioid receptors throughout the entire GI tract, treatment with laxatives displays relatively poor efficacy. Hence, a thorough understanding of GI physiology and opioid pharmacology is imperative to advance our understanding of OIBD.

The GI tract is innervated extrinsically by autonomic fibres from the CNS and intrinsically from the ENS (**Figure 2**). Exceptions include the striated muscle fibres in the oesophagus and the external anal sphincter, as these are innervated by spinal somatic fibres and somatic fibres from the pudendal nerve (S2-S3), respectively. The vagus nerve supplies parasympathetic innervation for the stomach, the small intestine, and proximal colonic segments from caecum to splenic flexure. The distal colonic segments, rectum and anal canal are parasympathetically innervated from the sacral roots (S2-S4)

(Rostgaard et al. 2006; Gudsoorkar & Quigley 2014). The stomach receives sympathetic innervation from the splanchnic thoracic nerves, which connect with postganglionic fibres from the coeliac plexus. The small intestine is primarily innervated by the superior mesenteric plexus and the distal colon is innervated by the inferior mesenteric plexus (Rostgaard et al. 2006).

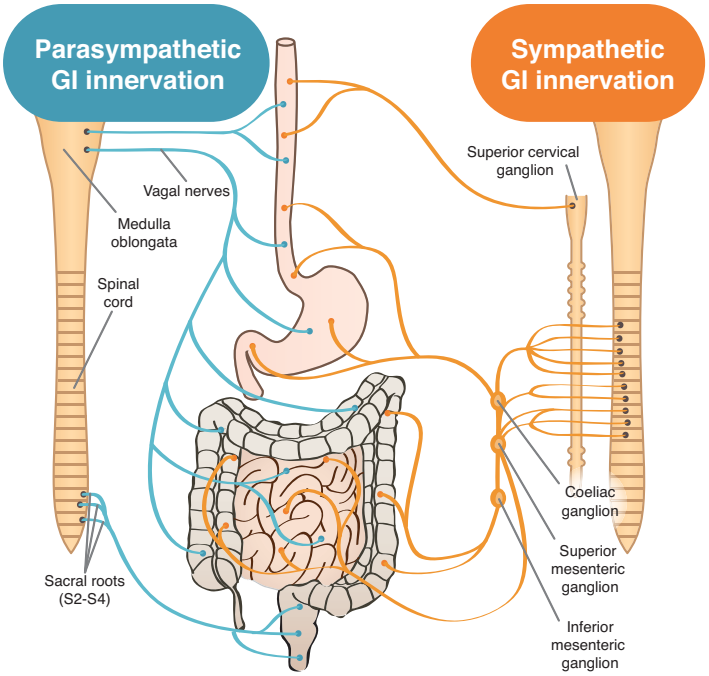

Figure 2: Schematic representation of the nervous innervation of the gastrointestinal tract.

While sympathetic stimulation reduces peristaltic activity and causes splanchnic vasoconstriction, parasympathetic stimulation increases peristaltic activity, secretion and vasodilatation. The primary excitatory neurotransmitters for the sympathetic and parasympathetic fibres are noradrenaline and acetylcholine. In effect, the afferents modulate local effector systems including musculature, secretory glands, and blood vasculature (Costa & Brookes 1994; Gershon 1981). These effector systems are controlled by motor neurons in the ENS transducing neural input originating from local sensory neurons, although some also receive input from the CNS via autonomic (both sympathetic and parasympathetic) pathways (Aziz & Thompson 1998).

The enteric nervous system is known as the 'brain of the gut' is comprised of some 200–600 million neurons and even more glia cells, placed in the gut wall along the entire GI tract (Furness et al. 2014). Here, they form complex interactions between sensory neurons, motoneurons, and interneurons exchanging information, constantly monitoring and controlling the GI effectors systems. The ENS motor neurons are divided into two principal types: the musculomotor neurons, which control the *muscularis externa* and the *muscularis mucosae* and the secretomotor neurons, which

innervate the different intestinal secretory glands (Wood 2010). The myenteric plexus is aptly named after its anatomical location between the longitudinal and the circular muscle layers along the GI tract as well as its control of the motor activity within the gut (**Figure 3**).

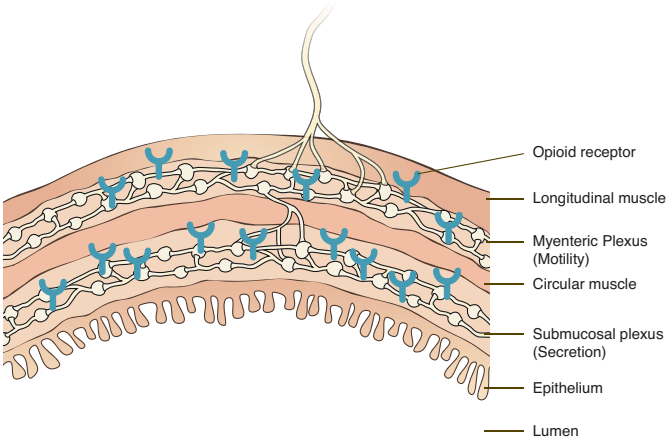

Figure 3: Schematic representation of the anatomical location of the myenteric and submucosal plexus in the gut wall.

The submucosal plexus is located closer to the gut lumen in the submucosa. Here, it controls gut secretion and gut absorption. Furthermore, the ENS possesses its own pacemaker system of cells of Cajal that ensures continuous gut peristalsis. The cells of Cajal are electrically coupled through gap junctions and generate oscillating 'slow wave' activity (Huizinga et al. 2013).

1.1.2 Opioid pharmacology

Within both the ENS and the CNS opioids exert their function through binding to specific opioid receptors. Four different receptors display affinity towards opioids, namely the μ-opioid receptor (MOR), the κ-opioid receptor (KOR), the δ-opioid receptor (DOR), and the opioid receptor like-1, which displays 65% sequence homology to the other receptors (Fioravanti & Vanderah 2008). The analgesic effect of opioids stems from binding to receptors within the CNS but the opioid receptors are widely distributed, both centrally and peripherally, as all receptors are synthesised within the dorsal root ganglia and from here, the receptors are transported via axons to nerve terminals in the periphery or in central structures (Epstein & Stein 1995).

The opioid receptors are G-protein coupled receptors and opioid binding to the receptor results in inhibition of voltage-gated ion channels, by impairing the enzymatic conversion of adenosine triphosphate to cyclic adenosine monophosphate (cAMP) (**Figure 4**). Cyclic AMP is an important regulator of normal cellular function and its inhibition leads to a decrease in the release of neurotransmitters such as glutamate, substance P, and calcitonin gene-related peptide (Trescot et al. 2008; Sharma et al. 1975). The resulting decrease in neuronal activity and release of

neurotransmitters alters both GI function via the ENS and pain perception via the CNS although evidence of cross-talk between these nervous systems exist (Galligan & Burks 1983; Thörn et al. 1996).

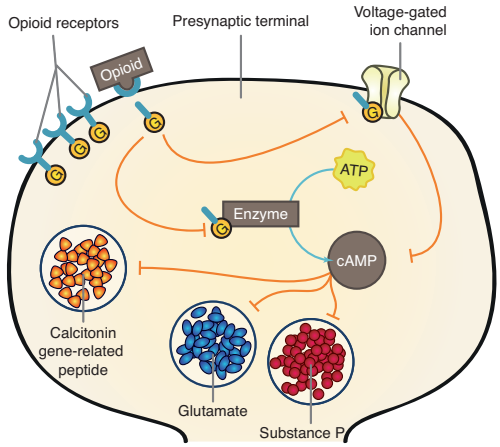

Figure 4: Simplified representation of the opioid mechanism of action at the presynaptic terminal. Opioid binding at the presynaptic terminal to G-protein coupled opioid receptors causes part of the G-protein to inhibit voltage-gated ion channels or to inhibit the enzymatic conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate via adenylate cyclase. Reduced ion influx further reduces the amount of intracellular cyclic adenosine monophosphate (cAMP). Ultimately, the overall effect is a reduction in the release of neurotransmitters including calcitonin-related peptide, glutamate, and substance P. Orange lines indicate inhibition.

From a clinical standpoint, the MOR is of particular importance because most commonly prescribed opioids display high affinity for the this receptor and because of its abundance in the GI tract (Sternini et al. 2004). In the human gut, MORs are primarily located on neurons in the myenteric and submucosal plexus and on mononuclear immune cells in the lamina propria where they are activated by endogenous ligands including enkephalins, endorphins, and dynorphins under physiological conditions (Greenwood-Van Meerveld et al. 2004; Sternini et al. 2004).

1.1.3 Gut motility

Gut motility is organised in a way that produces peristaltic contractions that 1) move ingested food in the form of a bolus from mouth to anus, 2) break up large food particles into smaller particles, 3) ensure adequate mixing of digestive enzymes with the bolus, and 4) allow sufficient mucosa-bolus contact for nutrient absorption. This motility is controlled from the myenteric plexus via neurotransmitters (e.g. acetylcholine, serotonin, vasoactive intestinal peptide, and nitric oxide) released from enteric neurons and excitation-contraction coupling in the circular smooth muscles. Acetylcholine activates the cholinergic excitatory motoneurons in the longitudinal smooth muscles, whereas nitric oxide and vasoactive intestinal peptide control the inhibition of non-cholinergic inhibitory motoneurons in the circular smooth muscles. Effectively, this allows the coordination of the

contractile and propulsive gut motility to be determined by a balance between facilitatory effects of acetylcholine and inhibitory effects of nitric oxide and vasoactive intestinal peptide (Wood & Galligan 2004; Sarna & Otterson 1990). As previously mentioned, the ENS has its own pacemaker system through networks of interstitial cells of Cajal. The oscillating slow wave activity generated by the interstitial cells of Cajal is independent of neural or hormonal input and the slow waves are conducted to muscle fibres in the circular and longitudinal muscle layers. Each muscle cell is individually activated by the pacemaker network through gap junctions because cell-cell propagation is not possible. Depolarization of the smooth muscle cells eventually opens voltage-gated calcium channels causing generation of action potentials, which ultimately leads to muscle contraction (Sanders et al. 2014; Sanders et al. 2012). The frequency of slow waves is different between GI regions and segments. The gastric antrum has a contraction frequency of approximately 3 contractions per minute (cpm), the duodenum 11–12 cpm, the proximal small intestine 11 cpm, which declines to 7-8 cpm distally, and the colon has 3-6 cpm. The contraction frequency in the GI tract does not increase beyond the pace set by the slow waves. Inhibitory neurons within the ENS can decrease the slow wave pace by preventing slow waves from causing a contraction. This also determines the length of the segment the contractions cover (Sanders et al. 2012; Wood 2009).

The ENS changes motility pattern based on the digestive state of the individual. Five to ten minutes after ingesting a meal, the postprandial state is activated, which lasts for the duration that food content remains in the stomach. Subsequently, the postprandial state cease and is replaced by the fasting state. During the fasting state, the migrating motor complexes (MMC) occur. The function of the MMC is thorough emptying of the stomach and small intestine, in order to prepare these for the next meal. While the ENS controls both motility patterns, the signal to switch from one to the other comes from the vagal nerve that detects distension of the stomach (Furness et al. 2014; Cassilly et al. 2008; Thomas 2008).

1.1.3.1 Opioid effects on motility

Opioid treatment alters oesophageal motility by inducing non-propulsive peristaltic contractions and incomplete relaxation of the lower oesophageal sphincter, which increases the risk of gastro-oesophageal reflux and dysphagia (Kraichely et al. 2010). Gastric emptying is prolonged during opioid treatment likely as a result of decreased gastric contractility (Rozov-Ung et al. 2014) while small intestinal and colonic effects of opioid treatment include increased resting contractile tone of the circular muscle layer coupled with a suppressed tonic inhibition of the muscle tone. Together, this causes an increased circular muscle tone (Frantzides et al. 1992; Telford et al. 1989; Sarna & Otterson 1990). Additionally, enhanced rhythmic contractions and high-amplitude non-propulsive phasic contractions is observed, which causes increased segmental spastic tone and reduced propulsive motility (**Figure 5**) (Thomas 2008; De Schepper et al. 2004; Kraichely et al. 2010). Clinically, these effects manifest as constipation, abdominal cramps, and bloating. These effects cause stasis of intestinal content, which prolongs the time for passive absorption of fluids. This naturally results in harder and drier stools that are difficult to pass.

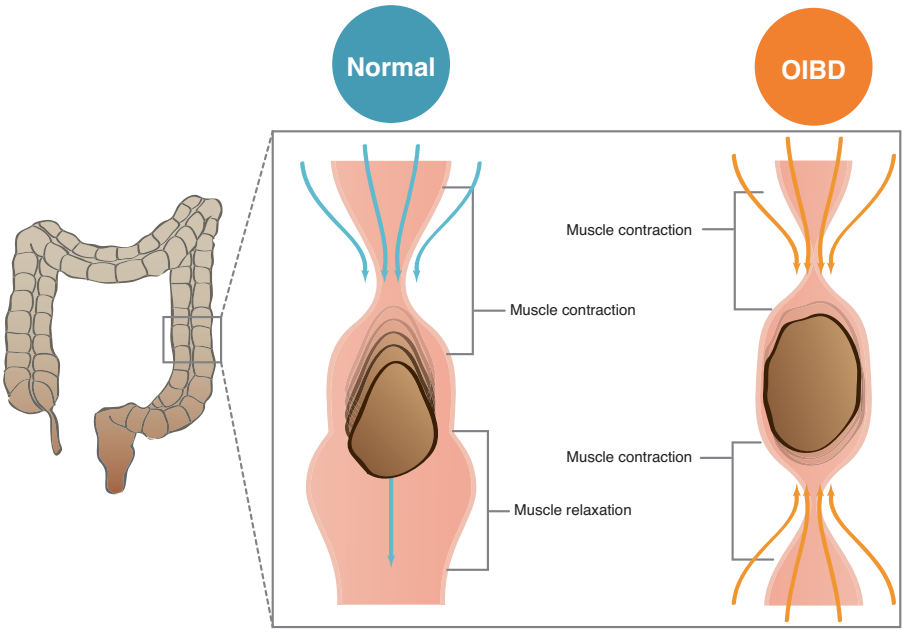

Figure 5: Schematic illustration of the dysfunctional gut motility during opioid-induced bowel dysfunction (OIBD). Under normal conditions propulsive movements occur through well-coordinated muscle contraction and relaxation. In OIBD there is an increased tonic muscle tonus and dyscoordinated muscle contractions that result in non-propulsive movements.

1.1.4 Gut secretion

Gut secretion is a pivotal factor in ensuring optimal conditions for digestion, absorption of nutrient, and propulsion of intestinal content. Every day the GI tract secretes an impressive volume of fluid of approximately 8-9 L (approximately 2 L saliva; 2.5 L gastric juice; 0.5-1 L bile; 1.5 L pancreatic juice and 1.5-2 L small intestinal secretions) (Barrett & Keely 2000; De Luca & Coupar 1996). In comparison, very little fluid is expelled with faeces under normal conditions, which means that the mechanisms controlling fluid and electrolyte secretion and absorption are closely regulated. Gut secretion relies on the osmotic gradient across the enterocyte because water cannot be actively secreted. To establish and control this gradient, several electrolytes are involved of which the most important are chloride, sodium, and bicarbonate. For example, active transport of chloride into the gut lumen will push the osmotic gradient to increase passive water transport into the gut lumen as well. Because chloride secretion is the major determinant of mucosal hydration excessive or insufficient secretion leads to conditions such as secretory diarrhoea or cystic fibrosis (Murek et al. 2010; Sidorov 1976).

One of the most prominent regulators of chloride secretion is the cystic fibrosis conductance regulator (CFTR). This is an apical cAMP-activated channel that regulate chloride secretion into the gut lumen either by direct phosphorylation of the regulatory R domain (chiefly by protein kinase A) and subsequent ATP hydrolysis at the nucleotide binding domain or by transporting additional CFTR

to the membrane (Figure 6) (Barrett & Keely 2000). The primary intestinal expression of CFTR occurs in the crypt and to a lesser extent in the villus itself. The pathogenesis of hereditary cystic fibrosis is based on mutations of the CTFR gene. Accordingly, patients with cystic fibrosis are predisposed to the development of chronic constipation (Grubb & Gabriel 1997).

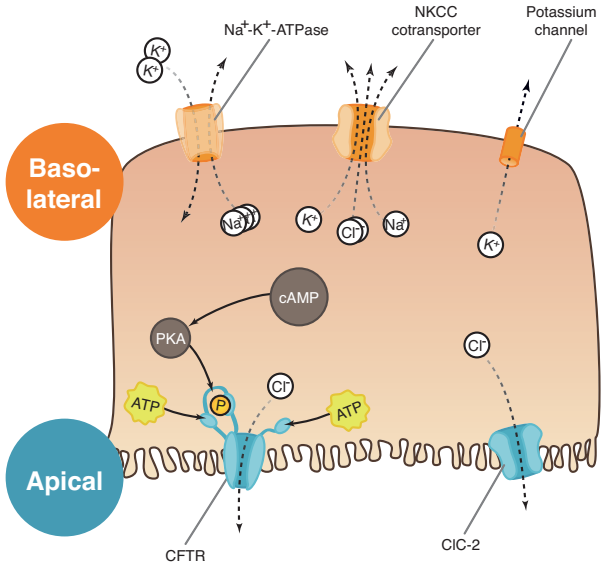

Figure 6: Important regulators of gut secretion. The principal chloride driver is the cystic fibrosis conductance regulator (CFTR). The CFTR is primarily activated by cyclic adenosine monophosphate (cAMP), which induces phosphorylation (P) of the regulatory R domain by way of protein kinase A (PKA) and subsequent ATP hydrolysis of the nucleotide binding domain. Another important chloride regulator is the chloride channel type-2 (ClC-2), which is also found in the apical enterocyte membrane. The Na^+-K^+-ATPase, the Na^+-K^+-$2Cl^-$ cotransporter (NKCC cotransporter), and potassium channels maintain a sustained favourable electronic gradient across the enterocyte.

The chloride channel type-2 (ClC-2) is another prominent chloride regulator. Primarily located on the apical surface of intestinal enterocytes ClC-2 its physiological contribution the small intestinal chloride secretion have yet to be determined. Knockout ClC-2 mice (-/-) do not suffer from neither GI obstruction nor increased mortality. Furthermore, double-knockout mice, where both genes for the CFTR and the ClC-2 were deleted did not exacerbate the symptoms observed with single knock-out of the CTFR gene (Zdebik et al. 2004). Nevertheless, Bijvelds and colleagues demonstrated evidence of cross talk between the ClC-2 and CFTR in a study investigating the effect of the selective ClC-2 agonist lubiprostone. Here, the secretory response to lubiprostone in tissue from healthy controls was compared to patients with cystic fibrosis. It was found that while lubiprostone significantly induced a secretory response in healthy tissue, it failed to do so in tissue with mutations to the CFTR (Bijvelds et al. 2009).

The chloride efflux into the gut lumen will gradually depolarise the enterocyte. However, this is kept in check by basolateral potassium channels and the Na^+-K^+-ATPase that counterbalance the chloride

efflux by maintaining a state of hyperpolarization (Mandel et al. 1986). Furthermore, the Na^+-K^+-$2Cl^-$ cotransporter ensures chloride basolateral chloride uptake to provide a substrate for sustained apical chloride secretion (Barrett & Keely 2000).

1.1.4.1 Opioid effect on secretion

During opioid treatment gut secretion is reduced as a direct result of inhibited cAMP and vasoactive intestinal peptide production. Subsequently, a general decrease in gut secretion of intestinal fluid secretion occurs, which leads to drier and harder stools. Furthermore, as gut motility is also dependent on volume inside the lumen via local stretch reflexes, decreased secretion (and volume) will also lead to a decrease in peristalsis (Furness & Costa 1987; Huizinga & Lammers 2009).

1.2 Treatment of OIBD

Satisfactory management of OIBD remains a challenge (Dorn et al. 2014; Bell et al. 2009). The current recommendation of combining laxatives with dietary changes and lifestyle changes is better suited for the treatment of e.g. chronic idiopathic constipation, where the underlying pathology is not due to opioid exposure. As it stands, this treatment strategy is often inadequate in the alleviation of OIBD and is even worsened because the majority of patients receiving chronic pain treatment suffer from co-morbidities resulting in e.g. less mobility (Diego et al. 2011; Dorn et al. 2014). Furthermore, past treatment of OIBD has revolved around normalization of spontaneous bowel movements (SBMs), leaving the remaining symptoms (e.g. straining, bloating, abdominal pain) unmonitored and untreated. The following sections are intended to provide an overview of the current treatment strategies and pharmacological approaches.

1.2.1 Laxatives

Laxatives are commonly prescribed to treat constipation and they generally exert their function by altering the composition and volume of the intestinal content, by stimulating gut motility, or through alterations of the ion and fluid transport across the intestinal epithelium. Accordingly, they are divided into different sub-groups based on their mechanism of action. These include the stimulant laxatives (e.g. bisacodyl, senna) that directly stimulate gut motility while inhibiting absorption of fluid and electrolytes from the gut lumen, which increases content volume. This in turn activates local stretch receptors and promotes further motility increase. The osmotic laxatives (e.g. magnesium, lactulose, polyethylene glycol) draw fluid into the gut lumen to increase peristalsis. An additional benefit is achieved from the increased luminal fluid in its stool softening effect. Electrolyte solutions with non-absorbable macrogols are laxative by way of their osmotic capacities. The electrolyte solutions are composed in a way that reduces fluid and electrolyte loss. The bulking agents (e.g. methylcellulose, psyllium) ease constipation by increasing the volume of stool and making it easier to pass. Finally, stool softeners are anionic surfactants that enable enhanced incorporation water and fats into the

stool, making it softer and traverse the GI tract with greater ease. Studies comparing different laxative regimens in patients with opioid-induced constipation are very limited and newer therapeutic agents are not routinely compared with established evidence-based treatment options, but rather to placebo. Although traditional laxatives have proven useful in inducing bowel movements, there is no convincing evidence to suggest which laxative is optimal for OIBD (Camilleri et al. 2014; Candy et al. 2011; Ahmedzai & Boland 2010). The few clinical trials comparing laxatives conclude that routinely used laxatives have comparable, suboptimal efficacy for opioid-induced constipation (Ruston et al. 2013; Agra et al. 1998; Freedman et al. 1997; Ramesh et al. 1998). This observation is supported by a study where chronic pain patients reported their bowel habits before and after initiating treatment with oral opioids with concomitant laxative use. Approximately half of the patients were using two or more laxatives. In the run-in 70% of patients reported ≥ 3 SBMs/week. After initiating oral opioid therapy, 55% reported having ≥ 3 SBMs/week yet interestingly, 81% still reported constipation as an opioid-induced adverse effect (Bell et al. 2009). This emphasises the point that monitoring of SBMs is an inadequate proxy for constipation and even more so for the multifaceted symptomatology of OIBD. The widespread application of SBMs as the primary outcome measure in previous studies also severely hinders comparison of new literature with existing in terms of the remaining clinical presentations of OIBD.

1.2.2 Chloride channel activator

Lubiprostone is derived from prostaglandin E1 and its mechanism of action relies on specific activation of the apical ClC-2 chloride channels in enterocytes (**Figure 6**) to improve stool consistency (Lacy & Chey 2009; Owen 2008). It was originally indicated for chronic constipation and constipation-predominant irritable bowel syndrome, where its efficacy was determined based on increased SBMs. Moreover, it was found that stool consistency, straining, bloating and severity of opioid-induced constipation improved as well (Wong & Camilleri 2011; Owen 2008). Subsequently, lubiprostone was approved in the US for treatment of opioid-induced constipation in adult patients with non-cancer pain where normal laxative treatment is inadequate (Camilleri et al. 2014; Mazen Jamal et al. 2012).

1.2.3 Selective 5-HT4 agonist

Prucalopride is a selective 5-HT4 agonist that alters colonic motility via serotonin 5-HT4 receptors in the gut. Primarily indicated and approved in many countries for chronic idiopathic constipation in females, but has demonstrated efficacy in opioid-induced constipation patients (Sloots et al. 2010). However, the effect was only significant at two weeks of treatment but not after four weeks and the drug is not approved for opioid-induced constipation. Furthermore, a randomised controlled trial comparing prucalopride to conventional treatment with macrogol in chronically constipated females found macrogol to be generally better tolerated and at least as efficacious as prucalopride (Cinca et al. 2013).

1.2.4 Tapentadol

Another approach to minimise the GI adverse effects of opioid treatment is through dual action drugs. One of these, tapentadol, is an opioid with classic MOR agonistic properties but with simultaneous action as a noradrenaline reuptake inhibitor. This dual action results in an additional analgesic effect (Tzschentke et al. 2009; Wade & Spruill 2009). It is efficacious in treating nociceptive and neuropathic pain conditions although data on its efficacy in the treatment of malignant pain is limited. The dual action also means that for an equianalgesic dose, less MOR agonism is required, which in turn improves the adverse effects profile (Afilalo & Morlion 2013). Accordingly, animal studies have shown less adverse effects on the CNS including nausea and vomiting from tapentadol use compared to equianalgesic doses of morphine (Tzschentke et al. 2009). In human trials, tapentadol compared to oxycodone exhibited improved GI tolerability and improved compliance with less treatment discontinuations (Wild et al. 2010; Buynak et al. 2010; Steigerwald et al. 2013; Wade & Spruill 2009).

1.2.5 Opioid antagonists

Where the other treatment strategies attempt to alleviate existing adverse effects or produce less adverse effects, opioid antagonists is a much more direct approach where the underlying pathophysiology is targeted specifically. Selective antagonism of MORs in the periphery should prevent the majority of all GI-related adverse effects. Several different drugs exist in this class and are distinguished primarily on grounds of their respective pharmacokinetic properties.

The archetype opioid antagonist is naloxone, which is a pure antagonist with no agonistic properties. Naloxone has been used widely as a highly effective antidote in the treatment of opioid overdose because of its very high affinity for the MOR. Given intravenously or intramuscularly, naloxone will antagonise both central and peripherally mediated opioid effects. Although oral naloxone undergoes extensive first-pass metabolism it is capable of crossing the blood-brain-barrier where it will reverse the central analgesic effects of opioid treatment. This is the primary reason for the absence of a stand-alone orally formulated naloxone product to treat OIBD (Vondrackova et al. 2008; Meissner et al. 2009).

Hence, successful use of opioid antagonists requires effective peripheral restriction. One attempt to achieve this property is based on the combination of prolonged release oxycodone and prolonged release naloxone in a 2:1 ratio tablet. The idea with this drug is to prevent OIBD from occurring through MOR antagonism in the periphery while preserving the analgesic effect of oxycodone in the CNS. The already low bioavailability of oral naloxone is further decreased by its relatively low dose and the fact that it is a prolonged release formulation, thereby rendering central antagonism unlikely (Smith et al. 2012). Studies have shown promising analgesic efficacy as well as improvement in OIBD symptoms (Burness & Keating 2014; Leppert 2013a; Leppert 2013b). In patients with hepatic impairment the bioavailability of naloxone may be enhanced because naloxone is metabolised in the liver (Leppert 2013a; Kraft 2008). Still, the primary drawback of this combination of prolonged release oxycodone and prolonged release naloxone is the fixed combination of oxycodone and naloxone in a

2:1 ratio in doses ranging from 5 mg oxycodone + 2.5 mg naloxone to 80 mg oxycodone + 40 mg naloxone. The fixed combination demands opioid rotation for patients treated with other opioids, and although recommendations are available this may be difficult outside specialist centres (Drewes et al. 2013).

Alvimopan is an oral peripherally acting MOR antagonist capable of increasing SBMs in patients with OIBD (Paulson et al. 2005; Roberts et al. 2002; Camilleri 2005). Because of cardiovascular safety concerns its development has been paused. However, the US Food and Drug Administration have approved the use of alvimopan in the treatment of post-operative ileus following partial bowel resection with primary anastomosis in hospitalised patients. Again, its applicability is of limited benefit to the general OIBD population because alvimopan is only registered in the US.

Naloxegol is a PEGylated naloxone moiety. Here, PEGylation of naloxone entails the attachment of a polyethylene glycol chain (Roberts et al. 2002). This chain is not capable of cross the blood-brain-barrier, which restricts naloxegol to the periphery where the naloxone part of naloxegol can act as a MOR antagonist (Webster et al. 2013). Naloxegol is administered orally once a day, and is advantageous in its ability be added to existing opioid therapy and thereby also allows for opioid rotation. It has proven efficacious compared to placebo on a number of different outcome measures, including OIBD symptoms and is generally well-tolerated with an acceptable safety profile (Chey et al. 2014; Webster et al. 2014; Bui et al. 2014).

Chapter 2 Hypotheses and aims

In order to describe the underlying pathophysiological mechanisms of OIBD in a controlled environment we developed an experimental model of OIBD in healthy volunteers (HVs) treated with prolonged-release oral oxycodone. It was hypothesised that assessments of gut function with 1) questionnaires, 2) MRI, 3) Ussing chambers, 4) the 3D-Transit system, and 5) FLIP would be sensitive in the detection of any alterations brought on by this experimental model of OIBD in HVs. Hence, the aims were:

I. *To describe the inter-individual and intra-individual variability of segmental colorectal MRI volumes between two observations in healthy subjects and the change in segmental colorectal volume distribution before and after defecation (paper I).*

II. *To investigate how oxycodone treatment, compared to placebo, affects sensitivity to somatic painful stimuli, bowel function assessed with questionnaires, and segmental colorectal volumes assessed with MRI (paper II).*

III. *To describe electrogenic epithelial ion transport in isolated mucosal biopsies from the rectosigmoid colon following five-day* in vivo *treatment with oxycodone compared to placebo and during* in vitro *addition of morphine (paper III).*

IV. *To evaluate how oxycodone treatment, compared to placebo, affects GI symptoms assessed by questionnaires and regional GI transit times using the 3D-Transit system (paper IV).*

V. *To evaluate how oxycodone treatment, compared to placebo, affects anal sphincter function and distensibility at rest and during challenge testing.*

Chapter 3 Materials and methods

The present dissertation is based on data from a single trial named MULTIPAIN6-2013 (sub-study 1b). The trial objective was to develop and validate a reliable model of experimentally induced OIBD in healthy volunteers where assessment of gut motility, gut secretion, and sphincter function were possible. These parameters are assessed by measuring 1) patient reported questionnaires, 2) segmental colorectal volumes using a novel MRI-based technique, 3) electrogenic epithelial ion transport in viable colonic tissue using Ussing chambers, 4) gastrointestinal transit times with the 3D-Transit system, and 5) anal sphincter function using FLIP.

The trial was approved by the local ethical committee of the Northern Jutland Region (N-20130030) and by the Danish Health and Medicines Authority (EudraCT no.: 2013-001540-60). The trial was covered by Danish Data Protection Agency under the umbrella approval of the Northern Jutland Region, registered with www.clinicaltrials.eu under the EudraCT number supplied above, and conducted in compliance with Good Clinical Practice (CPMP/ICH/135/95), designated Standard Operating Procedures, the Danish Health and Medicines Authority, the Research Ethics Committee in Denmark, and within the principles of the Declaration of Helsinki (amended by the 52nd General Assembly, Edinburgh, Scotland, October 2000, clarified by the General Assembly in Washington 2002, Tokyo 2004, and Seoul 2008 as outlined herein.

All subjects gave written informed consent prior to enrolment in the trial. Data was collected between April 2014 and February 2015 at the Mech-Sense research facilities at Department of Gastroenterology & Hepatology and Department of Radiology, Aalborg University Hospital, Aalborg, Denmark.

3.1 Study population

Twenty-five healthy male volunteers with neither history nor current symptoms of gastrointestinal disease were included in the study. All subjects underwent a screening session prior to enrolment in the study where a physician evaluated their medical history, ensured that all inclusion and no exclusion criteria were fulfilled, conducted a physical examination, and enrolled subjects if eligible.

Inclusion criteria were:

1) Signed informed consent declaration.
2) Capable of reading and understanding Danish.
3) Male of Northern European descent.
4) Understand what the study entails.
5) Aged 20-60 years.
6) Healthy.
7) Opioid naïve.

Exclusion criteria were:

1) Known hypersensitivity towards opioids.
2) Participation in any other studies within 14 days of enrolment.
3) Planned medical/surgical treatment within the study duration.
4) Need to operate heavy machinery or motor vehicles within the study duration.
5) Previous or current drug abuse.
6) Non-removable piercings or metal implants.
7) Daily alcohol consumption.
8) Daily nicotine consumption.
9) Known disease that may influence the results.
10) Use of prescription medicine and/or herbal medicine.

3.2 Induction of experimental OIBD

3.2.1 Study design and procedures

Sub-study 1b of MULTIPAIN6-2013 serves to validate the model of OIBD in healthy volunteers. It is designed as a two-armed randomised, double-blinded, placebo-controlled, crossover trial in 25 healthy volunteers. The subjects participated in two separate five-day periods, where they were randomised to either oxycodone or placebo in the first study period and then crossed over to the other treatment in the last period. A wash-out period of minimum 9 days was enforced to minimise the risk of a carry-over effect between treatment periods.

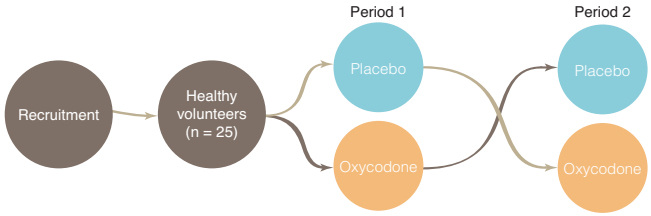

Figure 7: Trial design overview.

On day 1: Baseline assessments began with measurement of segmental colorectal volumes with MRI at the department of Radiology and when completed, the subjects returned to the Mech-Sense laboratory at the department of Gastroenterology and Hepatology, to complete the remaining experimental procedures. These included the following: Pain response to muscle pressure algometry, biopsy acquisition (for the Ussing chamber experiments), and all questionnaires. Subsequent to the completion of baseline measurements, the first dose of oxycodone/placebo was administered according to the randomisation procedure (**Figure 8**). The 3D-Transit capsule was swallowed and the second dose handed out for self-administration at a given time point. At home, the Bristol stool form scale (BSFS) questionnaire was continuously filled out every time the subject has a bowel movement.

A specific 3D-Transit diary was filled out continuously detailing the time points on which the subjects 1) had meals, 2) had bowel movements, 3) went to bed, 4) woke up, 5) changed battery, 6) watched TV, 7) used a computer, and 8) used transportation. The four first parameters were used in the data interpretation as they had the potential to affect gut motility. The four latter parameters were involved in data quality as battery changes produced loss of signal and TV/computer could interfere with electromagnetic noise while use of transportation could produce distinct movement artefacts.

On day 2: Pressure algometry, PAC-SYM questionnaire, and administration of the third dose. The fourth dose was handed out for self-administration at a given time point. At home, the BSFS questionnaire and 3D-Transit diary was filled out continuously.

On day 3: Pressure algometry, PAC-SYM questionnaire, and administration of the fifth dose. The sixth dose was handed out for self-administration at a given time point. At home, the BSFS questionnaire and 3D-Transit diary was filled out continuously.

On day 4: Pressure algometry, PAC-SYM questionnaire, and administration of the seventh dose. The eighth and ninth dose was handed out for self-administration at a given time point. At home, the BSFS questionnaire and 3D-Transit diary was filled out continuously.

On day 5: the healthy volunteers self-administered the ninth (and only) dose an hour before arriving at the research facility. Experimental procedures were performed: Pressure algometry, MRI, biopsy acquisition, and questionnaires.

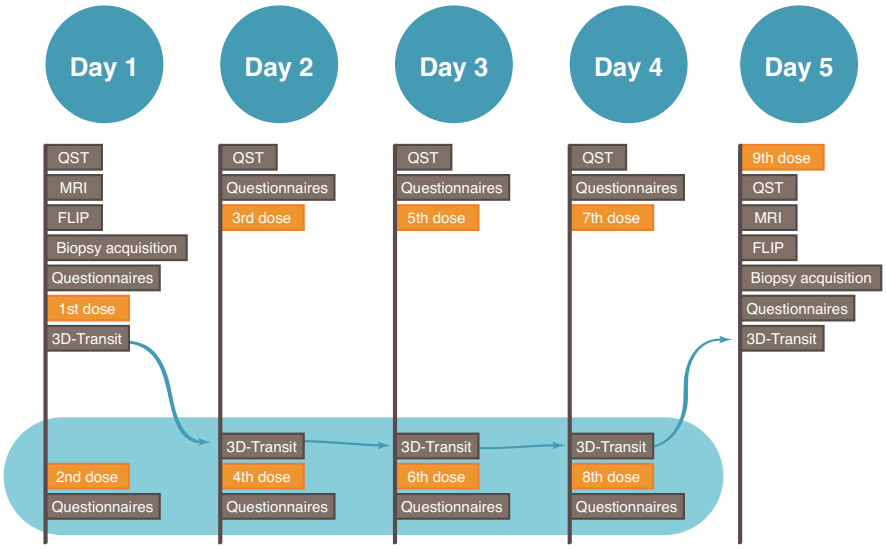

Figure 8: Experimental procedures for a given study period (Monday-Friday). The blue area in the bottom of the graph encircles procedures that took place out of the research facility. Subjects were instructed when to administer the 2nd, 4th, 6th, and 8th dose on day 1-4, and when to fill out the questionnaires by an automated text-message service. The 3D-Transit system was worn continuously until registration of capsule expulsion.

3.2.2 Study medication

Oxycodone is a semisynthetic opioid agonist, which is administered as a prolonged-release oral tablet, releasing oxycodone hydrochloride. It exerts agonistic effects on both peripheral and central opioid receptors. In controlled-release formulations it is used in cancer-related pain as well as chronic non-cancer-related pain problems (Riley et al. 2008). Due to its effect on the peripheral opioid receptors constipation is among the very commonly occurring adverse effects. Therefore, it was used mechanistically to induce OIBD. Placebo treatment was provided by Mundipharma Research GmbH & Co. KG and matched the physical appearance of prolonged release oxycodone. Treatment commenced with 5 mg twice daily on day 1. This dose was escalated to 10 mg twice daily on day 2 through day 4, and 10 mg once daily on day 5 (**Table 1**).

Table 1: Trial dose regimen.

Day	mg oxycodone/dose	Doses/day	Total daily dose
1	5 mg	2	10 mg
2	10 mg	2	20 mg
3	10 mg	2	20 mg
4	10 mg	2	20 mg
5	10 mg	1	10 mg

3.2.3 Pressure algometry

Pressure algometry was conducted within each day of all treatment periods in order to determine the analgesic effects of the administrated opioids. All stimuli were applied by the same examiner in order to improve consistency of the applied stimulus (Modir & Wallace 2010). Subjects were trained in reporting pain using a modified visual analogue scale (VAS - a continuous scale from 0-10 with anchor words for every increment of 1, that allows evaluation of both non-painful (from 0-5) and painful (from 5-10) sensation: 0=no sensation; 1=vague perception of mild sensation; 2=definite perception of mild sensation; 3=vague perception of moderate sensation; 4=definite perception of moderate sensation; 5=pain detection threshold (first time sensation was perceived as painful); 6=slight pain; 7=moderate pain; 8=medium pain; 9=intense pain; and 10=unbearable pain (**Figure 9**). The scale has been described in details previously and used extensively to assess pain intensity in several different tissues (Andresen et al. 2010; Drewes et al. 2003; Staahl et al. 2006).

Pressure was applied on the dorsal forearm with a handheld algometer (Type 2, Somedic production AB, Hörby, Sweden). The force increase rate was 30 kPa/s adjusted to a probe size of 1 cm^2. Two stimulations were applied per day on day 1 to day 5; first, the subjects were instructed to stop the stimulation when the stimulus quality changed from non-painful to painful (i.e. when the subjects reported 5 on the modified VAS). Second, the subjects were instructed to stop the stimulation upon reaching moderate pain (i.e. a 7 on the modified VAS). Pressure was applied at the

midpoint of the dorsal forearm to detect the pain threshold (VAS = 5) and 2 cm proximal to this point to detect moderate pain (VAS = 7). The two stimulations were separated by 10 seconds.

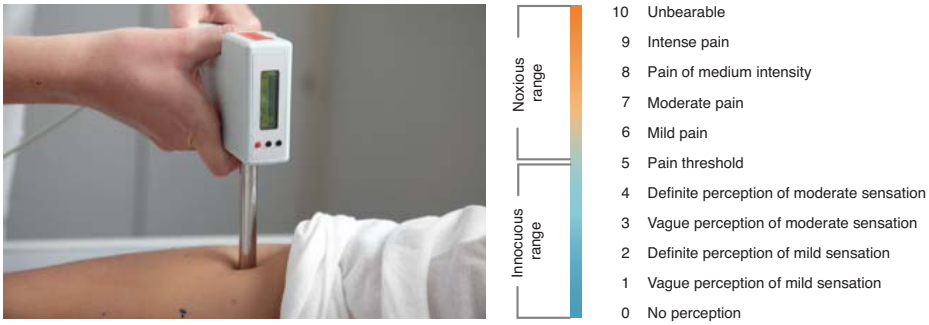

Figure 9: Quantitative sensory testing: Pressure was gradually increased on the muscles of the forearm until the subject rated the sensation VAS = 5 (pain threshold) and VAS = 7 (moderate pain).

3.3 Assessment of OIBD

The assessment of OIBD is complicated by its multifaceted symptomatology. Previous studies have relied heavily on constipation, focusing primarily on e.g. SBMs or transit times, which overlooks not only the remaining GI-related adverse effects, but also important aspects like patient focused perspectives as subjective severity and impact on quality of life. The importance of these additional aspects is emphasised by the fact that many opioid-treated patients report normal stool frequency, but still experience symptoms of OIBD (Bell et al. 2009). Therefore, a combination of subjective and objective assessment methods is recommended when evaluating OIBD.

3.3.1 Subjective assessments

3.3.1.1 Bowel function index (BFI)

The Bowel Function Index (BFI) is a three-item questionnaire that has been used to evaluate and assess the most frequently reported symptoms of OIBD (Rentz et al. 2009). It assesses the severity of 1) ease of defecation, 2) feeling of incomplete bowel evacuation, and 3) patients' personal judgment of constipation using a 0 to 100 numerical rating, where 0 = no problems and 100 = most severe problems. The main advantage of the BFI is the very brief form and precise questions. Furthermore, it is the only scale especially designed for opioid-induced constipation. Compared to other questionnaires the BFI tool is easy to use and consequently, missing data rarely occurs (<0.5%).

3.3.1.2 Gastrointestinal symptom rating scale (GSRS)

The Gastrointestinal symptom rating scale (GSRS) is composed of 15 items (epigastric pain, epigastric burning, gastro-oesophageal reflux symptom, belching, postprandial fullness, abdominal distension, early satiety, abdominal pain, the feeling of hunger, nausea, intestinal rumbling,

constipation, diarrhoea, loose stools and hard stools), which comprise five dimensions including gastro-oesophageal reflux, abdominal pain, dyspepsia, diarrhoea, and constipation. Each item is rated according to severity on a seven-point graded Likert-type scale, where 1 = absence of troublesome symptoms and 7 = very troublesome symptoms. The reliability and validity of the GSRS are well-documented (Dimenäs et al. 1995), and normal values for a general population are available (Dimenäs et al. 1996).

3.3.1.3 Patients assessment of constipation symptom scores (PAC-SYM)

The PAC-SYM is comprised of 12 items assigned to three subscales: stool symptoms, rectal symptoms, and abdominal symptoms. Each symptom is rated on a 5-point Likert scale. In addition, the PAC-SYM also contains a question about the frequency of the bowel movements during the past 7 days. Frank et al. found internal consistency and that test-retest reliability of the final instrument was high. Both patients and investigators can rate severity of constipations, and these ratings by different groups were also found to be correlated (Frank et al. 1999). The PAC-SYM is internally consistent, reproducible under stable conditions, valid, and responsive to change, and provides a comprehensive means to assess the effectiveness of treatment for constipation or for monitoring the symptoms of opioid-induced constipation (Frank et al. 1999; Slappendel et al. 2006). Therefore, it may be used in clinical settings to assist with the disorder management, bridging the gap between clinical standards and non-systematic self-reports (Frank et al. 1999).

3.3.1.4 Spielberger's State-Trait Anxiety Inventory (STAI)

The questionnaire is used the level of anxiety within each subject (Hodges & Spielberger 1969). This questionnaire stands out as the most effective way of measuring an individual's anxiety level as it clearly differentiates between temporary 'state anxiety' and more general long-term quality of 'trait anxiety'. The state anxiety section of this questionnaire allows for quantification of anxiety levels of a given subject at a particular point in time. Furthermore, the STAI questionnaire enables detection of differences in anxiety levels over time. Both STAIS and STAIT consist of 20 positive and negative item such as 'I feel calm' or 'I am worried' that the subject rates from 1 = not at all to 4 = very much so.

3.3.1.5 Subjective Opiate Withdrawal Scale (SOWS)

The Subjective Opioid Withdrawal Scale is a self-administered scale for grading opioid withdrawal symptoms. It contains 16 items (symptoms) whose intensity the patient rates on a scale of 0 = not at all to 4 = extremely. This scale is demonstrated to be a valid and reliable indicator of the severity of the opiate withdrawal syndrome over a wide range of common signs and symptoms (Handelsman et al. 1987).

3.3.2 Objective assessments

3.3.2.1 The Bristol stool form scale (BSFS)

The Bristol Stool Form Scale is an objective assessment of the most frequently reported OIBD symptom: constipation. It can partly detect parameters like stool frequency and stool consistency and is used as a proxy of intestinal transit time (Lewis & Heaton 1997). However, the BSFS shows only a moderate correlation between stool form and measured whole gut or colonic transit time in constipated adults. Moreover, stool frequency is a poor surrogate for transit, even in those with reduced stool frequency (Saad et al. 2010). In fact, determination of stool form provides a qualitative rather than quantitative assessment of transit.

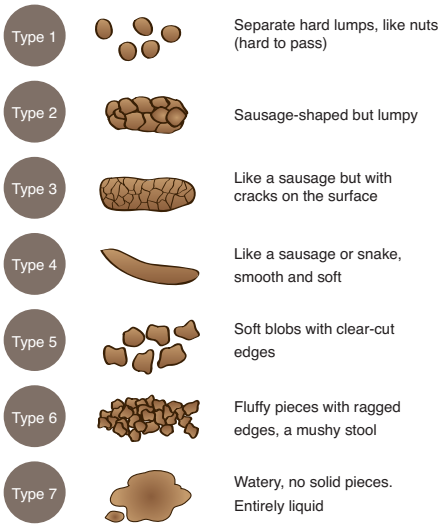

Figure 10: Bristol stool form scale.

3.3.2.2 Assessment of colon volume (MRI)

Assessment of gastrointestinal volumes with MRI has been applied in different contexts previously (

Table 2). Aside from context these studies also vary in methodology in terms of obtaining quantifiable volumes from MRI scans. Some studies were based on completely manual segmentation, which is both time consuming and can be subject to a great deal of observer bias. Others are semi-automatic, which often reduces time consumption and observer bias, but due to the similarity in image intensity of GI organs and surrounding tissues, a fully automatic method is very difficult to develop.

In relation to the present dissertation, a novel semi-automatic method has been developed from 2012-2014 in collaboration with Aalborg University, Department of Health Science and Technology. The software has been dubbed “Colometry v. 1.0” and has demonstrated good performance in terms of inter-observer reliability (Sandberg et al. 2015). Segmental colorectal volumes of the caecum/ascending colon, transverse colon, descending colon, and rectosigmoid colon can be determined through manually placed regions of interest on the T2-weighted scans. These regions of interest are used to outline the four colon segments in each of the 35-40 coronal slices per scan. Within these rough outlines the software is capable of determining the exact outer boundaries of the colon automatically, because both colon lumen and gut wall appear dark on T2-weighted scans, compared to the bright signal of adjacent organs and abdominal fat.

Colon anatomy varies substantially between subjects and in order to secure consistency, each region was segmented according to a simple set of guidelines: 1) The caecum/ascending colon included the caecum to the hepatic flexure; 2) the transverse colon is the segment between hepatic flexure and splenic flexure; 3) transition from descending colon to sigmoid colon exhibited the greatest inter-individual variability (due to the absence of any clear/consistent anatomical bend of the colon) and was subsequently subject to the greatest discrepancies in manual segmentation. This was resolved by implementing a feature to the Colometry software placing a horizontal guideline that intersected the left anterior iliac spine. This line was used as the boundary between the descending and sigmoid colon. Hence, the descending colon was defined between splenic flexure and horizontal guideline, whilst 4) the rectosigmoid colon was defined between horizontal guideline and anus. The observer requires approximately 20 minutes to define the regions of interest for all 35-40 contiguous slices in an examination and the subsequent determination of volumes by the Colometry software is completed in 2-3 minutes.

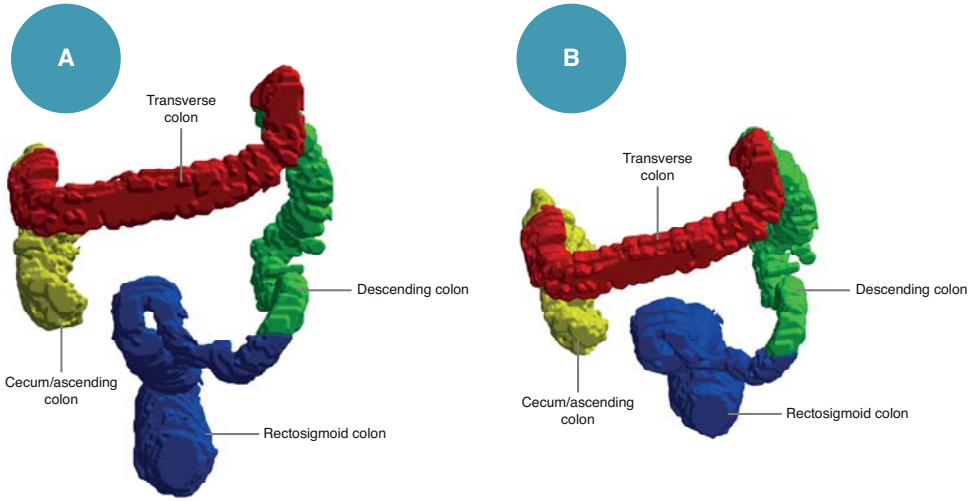

Figure 11: Two angles of the same fully segmented abdominal MRI scan series with the Colometry v. 1.0 software. (A) Frontal view. (B) Frontal view with forward tilt.

Table 2: List of studies on MRI-based determination of gastrointestinal (GI) volumes listed in descending order based on year of publishing.

Authors	Population	GI segments	Automation	Context
(Murray et al. 2015)	18 HVs	Stomach	Manual	Effect of aerated drinks on gastric distension.
(Sandberg et al. 2015)	4 HVs	Colon	Semi-automatic	Inter-observer reliability of semi-automatic method.
(Pritchard et al. 2014)	75 HVs 25 IBS-D patients	Colon	Manual	Postprandial effect on colon volumes.
(Bharucha, Fidler, et al. 2014)	43 HVs	Stomach, small intestine, colon	Manual + Semi-automatic	Effect of erythromycin on gastric emptying.
(Bharucha, Karwoski, et al. 2014)	20 HVs 29 dyspeptic patients	Stomach	Semi-automatic	Comparison of semi-automatic technique with manual techniques.
(Murray et al. 2014)	16 HVs	Stomach, small intestine, colon	Semi-automatic	Impact of fructose and fructans ingestion on GI volumes.
(Marciani et al. 2014)	24 HVs	Stomach, small intestine, colon	Manual + Semi-automatic	Effect of PEG dosing on GI volumes.
(Placidi et al. 2012)	18 HVs	Stomach, small intestine, colon	Semi-automatic	Effect of loperamide on gut water distribution in a mannitol model of secretory diarrhoea
(Di Palma et al. 2011)	12 HVs 20 IBS patients	Rectum	Manual	Comparison of rectal volume in IBS patients vs. HVs.
(Rubesova et al. 2009)	83 foetuses	Colon	Manual	Volume of the normal foetal colon.

HVs: healthy volunteers; IBS: irritable bowel syndrome; IBS-D: diarrhoea-predominant irritable bowel syndrome.

3.3.2.3 Assessment of gut secretion (Ussing chamber)

The Ussing chamber is a powerful, yet simple technique to assess ion transport across tissues. Several studies have investigated human colonic biopsies and ion transport (Wallon et al. 2005; Kaltoft et al. 2010; Kleberg et al. 2012). However, the literature is very limited on the effects of opioids on gut secretion in human tissue (

Table 3). The studies that do exist investigate the effect of direct opioid application to intestinal tissue *in vitro* (Sun et al. 2011; Burleigh 1991), while no studies exist where human subjects are treated with opioids *in vivo* with subsequent Ussing chamber experiments on isolated tissue. Hence, in the present context we wish to further our understanding of the underlying mechanisms of opioid-induced alterations in gut secretion and to evaluate the contribution of decreased gut secretion during oxycodone treatment to the collective development of OIBD. To do so, a series of compounds were added to extracted mucosal biopsies with either a known stimulatory effect (prostaglandin E_2 (PGE_2) and theophylline) or an inhibitory effect (ouabain and possibly morphine). These compounds have previously been used to investigate specific secretory mechanisms. For example, addition of PGE_2 increases the amount of intracellular cAMP and theophylline inhibits enzymatic degradation of cAMP by phosphodiesterase (Larsen et al. 2005; Hansel et al. 2004; Kaltoft et al. 2010). Therefore, these two compounds should exert a pro-secretory effect by increasing the available amount of cAMP, which in turn can activate the CFTR channels. Activation of CFTRs increases the efflux of chloride ions, thereby increasing gut secretion (Barrett & Keely 2000). On the other hand, ouabain inhibits the Na^+-K^+-ATPase, which reduces the secretory capacity by limiting the ability of the enterocyte to maintain a favourable osmotic gradient (Dharmsathaphorn et al. 1985). The ability of morphine to limit gut secretion is likely not through a direct effect on gut mucosa and was added to substantiate this hypothesis (Burleigh 1991; Sun et al. 2011). In the present work these specific cellular secretory mechanisms are assessed at baseline and after oxycodone treatment and placebo treatment in order to determine whether oxycodone treatment induces alterations to any of these mechanisms. This will provide valuable information on the underlying mechanisms by which gut secretion is altered during opioid treatment.

Within each study period, biopsies were taken from the rectosigmoid junction as this location was easily identified by an abrupt turn into the left lower quadrant (this location was found 15-20 cm from the anal verge, depending on inter-individual anatomical variation). Biopsies were taken at baseline before starting treatment and after five days of treatment. Four biopsies were taken per subject per day to increase the likelihood of obtaining at least one successfully mounted, viable and responsive biopsy at each time point. Biopsies were extracted in close proximity to each other (within five cm^2). Slight scarring with granulation from the baseline biopsy extraction remained visible on day 5 in most cases and was used to ascertain biopsy extraction from a representative adjacent site.

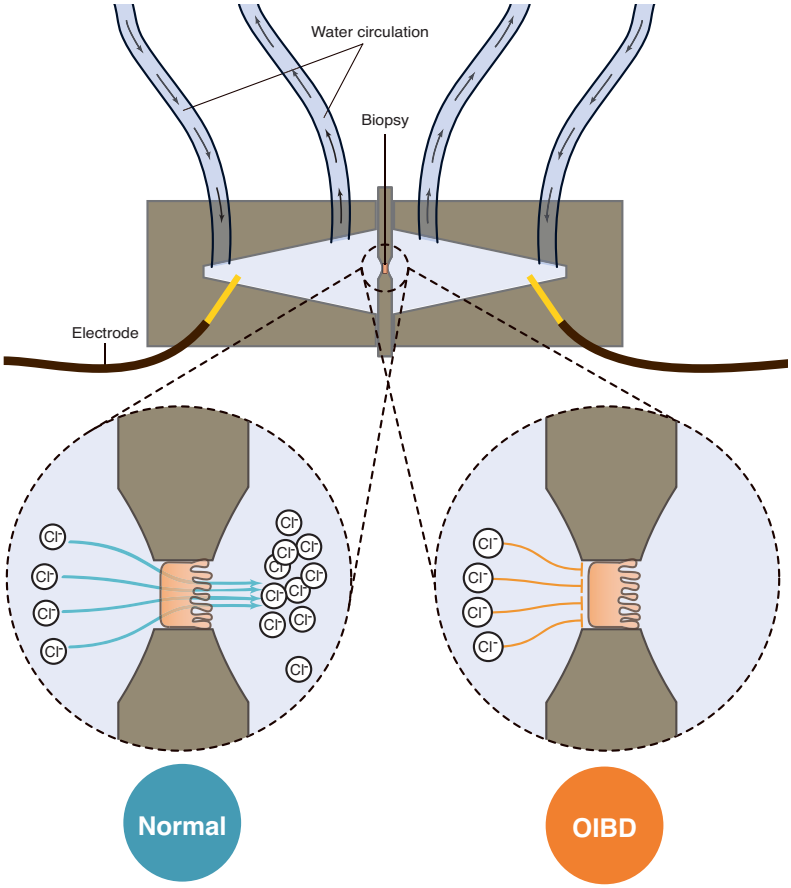

Figure 12: Schematic representation of the Ussing chamber setup. A biopsy is mounted between two chambers that simulate the blood stream on the basolateral side of the biopsy and gut lumen on the apical side. The electrogenic epithelial ion transport can be measured across the biopsy. It was assumed that the chloride transport would decrease during OIBD.

Table 3: Ussing chamber studies on opioid effect listed in descending order based on year of publishing.

Authors	Population	GI segments	OR interaction	Results
(Sun et al. 2011)	Human	Jejunum	-	Morphine suppresses basal SCC and lubiprostone can reverse this effect.
(Fei et al. 2010)	Guinea pig and mouse	Ileum, colon	MOR	Morphine suppresses basal SCC and lubiprostone can reverse this effect.
(Fichna et al. 2009)	Mouse	Colon	KOR	Salvinorin A suppresses colonic secretion.
(Schreiber et al. 2004)	Mouse	Colon	KOR	Asimodoline suppresses colonic SCC.
(Green et al. 2003)	Pig	Ileum	DOR	Kallidin increases ileal SCC.
(Yates et al. 2001)	Rat	Colon	-	Stress tests increase SCC and the effect can be reversed with naloxone.
(Poonyachoti & Brown 2001)	Pig	Ileum	DOR	Mast cell degranulation increases SCC and the effect can be reversed with a DOR agonist.
(Greenwood-Van Meerveld et al. 2000)	Rat	Jejunum, colon	-	Wood creosote and loperamide does not affect normal SCC but can reduce SCC under hyper-secretory conditions.
(Gauthier & Reddix 2000)	Guinea pig	Colon	DOR	DOR agonism can both supress and increase basal SCC depending on dose.
(Poonyachoti & Brown 1999)	Pig	Ileum	DOR	Selective DOR agonism potentiates secretory effects of hypersensitivity.
(Kromer 1995)	Guinea pig	Colon	MOR	Loperamide has pro-secretory actions at the MOR.
(Burleigh 1991)	Human	Colon	-	Loperamide but not morphine has anti-secretory effects in human colon.
(Sheldon et al. 1990)	Mouse	Jejunum	DOR, KOR, MOR	Agonists for all ORs can suppress basal SCC and increase G.
(Kachur et al. 1980)	Guinea pig	Ileum	DOR	DOR agonists reduce SCC. MOR agonism with fentanyl and morphine is ineffective.

GI: gastrointestinal; OR: opioid receptor; SCC: short circuit current; MOR: μ-opioid receptor; KOR: κ-opioid receptor; DOR: δ-opioid receptor.

3.3.2.4 Assessment of gut motility (3D-Transit system)

The 3D-Transit system is a minimally invasive and non-radiant tool, which relies on a small wireless telemetric capsule that is ingested and extracorporeal portable detector with four sensors that continuously track the capsule's location as it traverses the GI tract (**Figure 13**). Each capsule (dimensions: 21 mm x 8 mm, density 1.6 g/cm^3) is comprised of an electromagnet and a battery. Battery life of the capsule is 120-180 hours at 5 Hz sampling frequency.

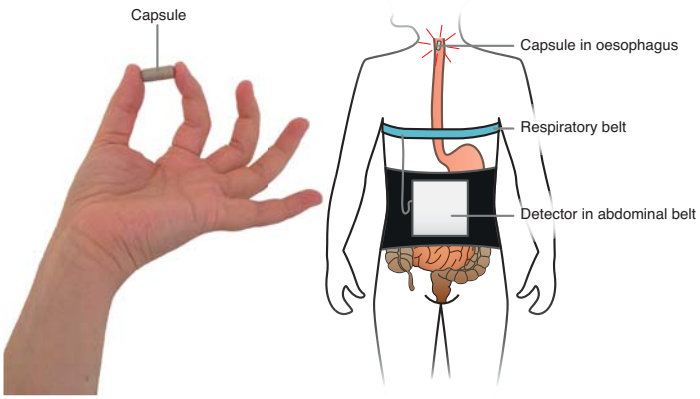

Figure 13: The 3D-Transit system. The small capsule is easily swallowed and the monitoring system can be worn discretely underneath the test subjects' own clothes for the duration of the monitoring period.

As the capsule traverses the GI tract, the electromagnetic field emitted by the capsule is stored on a memory card in the detector. Upon completion of the examination the data stored on the memory card is converted into space-time coordinates (x;y;z;Φ;θ) through an iterative algorithm. The x;y;z coordinates refer to the distance between detector and capsule in the frontal, transversal, and sagittal plane, while the Φ;θ coordinates refer to the angular orientation of the capsule.

Artefacts due to breathing and postural changes are recorded with an elastic sensor belt that is fixated around the subject's chest. Movement artefacts are recorded by an accelerometer within the detector.

Ultimately, the system yields provides a valid measure of gastric, small intestinal, and colonic transit (Worsøe et al. 2011). This magnetic capsule is swallowed and the mobile tracking system can be worn at home allowing healthy volunteers and patients to leave the research facility, whilst the system measures continuously. Because the gastric transit is largely dependent on when the subject has eaten, it is important for this measure that all healthy volunteers are in the fasting state when they arrive the research facility on day 1. Here, they ingested a small standardised meal consisting of three muesli bars and a cup of tap water followed by ingestion of the 3D-Transit capsule. Following the standardised meal the subjects were instructed to fast for another six hours. After these six hours, the subjects' eating habits were not controlled further. Previous studies investigating the effect of opioid treatment on regional colonic motility or transit times are listed in **Table 4**.

Table 4: Studies investigating the effect of opioid treatment on regional gastrointestinal transit times listed in descending order based on year of publishing.

Authors	Population	GI segments	Method	Results
(Rozov-Ung et al. 2014)	15 HVs	Stomach	Wireless motility capsule	Opioid treatment prolongs GE
(Jeong et al. 2012)	38 HVs	Stomach, small intestine, colon	Scintigraphy	Opioid treatment prolongs GE and SBT but not CT
(Yancey-Wrona et al. 2011)	10 HVs	Orocecal transit time	Breath test	Opioid treatment prolongs orocecal transit time.
(Smith et al. 2011)	15 HVs	Orocecal transit time	Scintigraphy	Opioid treatment prolongs orocecal transit time
(Gonenne et al. 2005)	74 HVs	Stomach, small intestine, colon	Scintigraphy	Opioid treatment prolongs GE, SBT, and CT
(Freye & Latasch 2000)	12 HVs	Orocecal transit time	Breath test	Opioid treatment prolongs orocecal transit time
(Maurer et al. 1996)	12 HVs	Stomach, small intestine, colon	Scintigraphy	Opioid treatment does not affect GE, and SBT but prolongs CT
(Yee et al. 1991)	10 HVs	Stomach, small intestine	Scintigraphy, breath test, time to first flatus	Opioid treatment prolongs GE, SBT, and time to first flatus
(Thorén et al. 1989)	9 HVs	Stomach, small intestine	Scintigraphy, breath test	Opioid treatment prolongs GE and SBT
(Prokop et al. 1988)	10 HVs	Stomach, small intestine	Scintigraphy, breath test	Opioid treatment prolongs GE and SBT
(Kaufman et al. 1988)	12 HVs	Colon	Scintigraphy	Opioid treatment prolongs CT.

GI: gastrointestinal; HVs: healthy volunteers; GE: gastric emptying; SBT: small bowel transit; CT: colonic transit.

3.3.2.5 Manometry and impedance planimetry (FLIP)

Sphincter (dys)function and dyscoordination is complex and difficult to assess. Anorectal manometry is the most common technique for assessment of anorectal dysfunction such as impaired rectal contraction, paradoxical anal contraction, impaired anal relaxation, or a combination of these conditions. Other possible tests are defecography or the balloon expulsion test, which provides assessment of the patient's ability to defecate (Rao 2007). However, in biomechanical terms the overall ability of a sphincter to distend or open is related to many factors including muscle tone, passive viscoelasticity, mechanoreceptor-mediated reflexes and perception, none of which can be measured sufficiently with the previous mentioned methods (Gregersen 2002). To overcome problems like this, methods such as the Functional Lumen Imaging Probe technique (FLIP) have been developed to study GI sphincters (McMahon et al. 2005). The main advantage is that it can distinguish between an open and closed sphincter and record the distensibility during challenge tests (i.e. squeezing, expulsion), whereas manometry only shows the difference between a toned and a relaxed sphincter (McMahon et al. 2006; Alqudah et al. 2012). Thus, the FLIP may provide useful information in the evaluation of anorectal dysfunction in OIBD patients.

The functional lumen imaging probe (FLIP) is a device, which is capable of measuring the cross-sectional area within a cylindrical bag, which is filled with electrolyte-enriched water. Within the cylindrical bag, a thin probe with 12 electrodes measure the distance to the bag and calculate the cross-sectional area in real time. On the recording unit the data output is seen in real-time. In the present trial the FLIP was positioned in the anal sphincter and filled with 10 mL of water. Correct positioning in the anal sphincter was seen as a characteristic hour glass-shape as shown in **Figure 14** because the infused water will fill the bag in area of least resistance (which in this case is on either side of the anal canal). Once proper positioning is ascertained, the FLIP is fixated manually during the actual assessment, which consists of two parts: 1) sphincter distensibility at rest and sphincter function during challenge testing. Sphincter distensibility at rest was carried out by infusing the bag in a stepwise manner with 10 mL, 30 mL, and 50 mL of water. At each step the sphincter was measured for 30 seconds. Sphincter function during challenge testing was carried out by infusing the bag with 10 mL, 30 mL, and 50 mL once more but this time the subjects were instructed to actively exert pressure on the bag. This was done by squeezing with the external anal sphincter as much as possible for 10 seconds at each step. This method provides information on the coordination of the opening- and closing dynamics of the sphincter (Drewes 2009).

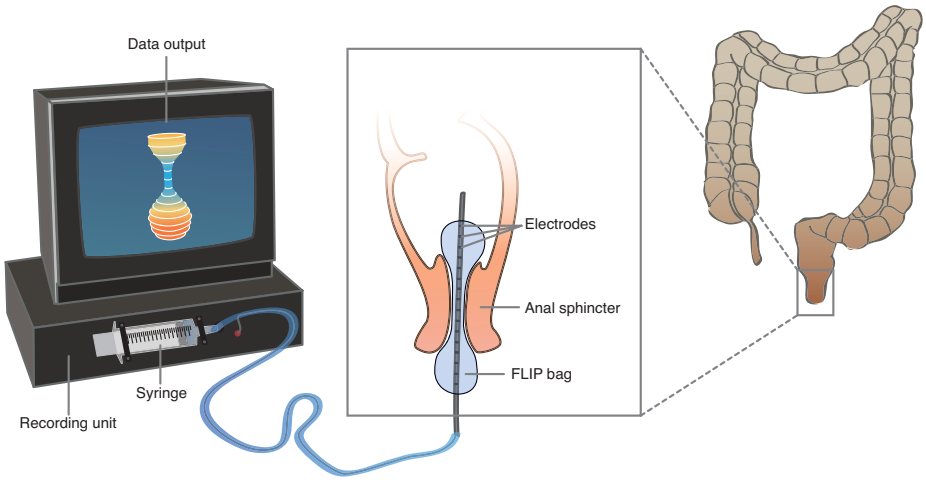

Figure 14: The FLIP system. A bag is connected to a computer-controlled syringe with water. The probe measures the cross-sectional area of a specific GI segment (in this case, the anal sphincter).

3.4 Justification of sample size

As this is an exploratory study accurate estimations of sample size can be difficult. Nevertheless, the sample size was based on the whole gut transit time measured with the 3D-Transit system, as this was the primary endpoint. A similar study previously investigated the same endpoints with the 3D-Transit system in patients with neuroendocrine tumours (Gregersen et al. 2011). Here, 12 patients were included to estimate the effect of sandostatin on GI transit time. A mean difference of 0.47 days in total transit time at the end of treatment was estimated with a variance of 0.37, equivalent to a standard deviation (SD) of 0.61 and the effect of opioid treatment on GI transit time was expected to yield at least similar results. Performing power calculation to estimate a rational sample size we use a mean difference of 0.5 days in total transit time and a variance of 0.4. With a power of 95% and α at 0.05 in a 2-sided t-test, the calculation estimates a sample of 19 subjects as appropriate. Since a difference of 0.5 days in total GI transit time may be of clinical relevance and expected to be feasible to obtain with the treatment, we suggest aiming for a sample of 20 subjects to obtain 95% power. Therefore, 25 subjects will be included in order to increase the likelihood of having sufficient complete data sets.

Chapter 4 Results

The key results from the studies are presented in this chapter. More detailed results are found in paper I-IV.

4.1 Aim I

Aim: To describe the inter-individual and intra-individual variability of segmental colorectal MRI volumes between two observations in healthy subjects and the change in segmental colorectal volume distribution before and after defecation (paper I).

Key results:

- No significant differences between the two observations were detected for any segments (All $P>0.05$).
- Inter-individual variability varied across segments from low correlation in caecum/ascending colon (intra-class correlation coefficient (ICC)=0.44) to moderate correlation in the descending colon (ICC=0.61), and high correlation in the transverse (ICC=0.78), rectosigmoid (ICC=0.82), and total volume (ICC=0.85).
- Overall intra-individual variability was low (coefficient of variance=9%).
- After defecation the volume of the rectosigmoid decreased by 44% ($P=0.003$). The change in rectosigmoid volume was associated with the true faecal volume ($R^2=0.72$, $P=0.02$).

Interpretation: The caecum/ascending colon exhibited the most variability, potentially reflecting its capacity to receive and accommodate content arriving from the small intestine and store it until moved distally by a mass-movement. Accordingly, the inter-individual variability was lower for the remaining colorectal segments. Imaging of segmental colorectal volume, morphology, and faecal accumulation is advantageous to conventional methods in its low variability, high spatial resolution, and its absence of contrast-enhancing agents and irradiation. Hence, the method is suitable for future clinical and interventional studies as well as for characterisation of defecation physiology.

4.2 Aim II

Aim: To investigate how oxycodone treatment, compared to placebo, affects sensitivity to somatic painful stimuli, bowel function assessed with questionnaires, and segmental colorectal volumes assessed with MRI (paper II).

Key results:

- Compared to baseline, oxycodone increased pain detection thresholds by 8% ($P=0.02$).

- Oxycodone treatment induced OIBD seen as increased scores in the BFI questionnaire (464% increase; $P<0.001$), the GSRS questionnaire (37% increase; $P<0.001$), and the PAC-SYM questionnaire (198% increase; $P<0.001$) compared to placebo.
- Objectively, stools were harder and drier during oxycodone treatment ($P<0.001$) and colorectal volumes also increased (caecum/ascending colon by 41% ($P=0.005$) and transverse colon by 20% ($P=0.005$)) compared to placebo.
- No association between questionnaire scores and colorectal volumes were detected.

Interpretation: The oxycodone treatment regimen was sufficient to induce analgesia and experimental OIBD in healthy volunteers. The MRI-based method was sensitive to changes in colorectal volumes brought on by oxycodone treatment and the OIBD model itself shows potential for future interventional studies to discriminate efficacies of different laxative in combination with opioid treatments.

4.3 Aim III

Aim: To describe electrogenic epithelial ion transport in isolated mucosal biopsies from the rectosigmoid colon following five-day in vivo *treatment with oxycodone compared to placebo and during* in vitro *addition of morphine (paper III).*

Key results:

- No difference in basal SCC or basal G was found between any study days (all $P > 0.05$).
- Addition of secretagogues (PGE2 and theophylline) produced similar baseline-corrected ΔSCC and ΔG during oxycodone and placebo treatments ($P>0.05$).
- Addition of inhibitors (morphine and ouabain) produced similar baseline-corrected ΔSCC and ΔG during oxycodone and placebo treatments ($P>0.05$). In fact, morphine addition did not produce any response to ΔSCC and ΔG at all.

Interpretation: In vivo *oxycodone treatment in healthy volunteers does not alter electrogenic epithelial ion transport in the sigmoid colon. Neither does* in vitro *application of morphine to sigmoidal biopsies alter the electrogenic epithelial ion transport, evidently because of the absence of MORs in human colonic mucosa. As the secretory capacity and structural integrity appears intact in this segment despite oxycodone treatment it suggests that oxycodone treatment does not affect gut secretion in the distal colon.*

4.4 Aim IV

Aim: To evaluate how oxycodone treatment, compared to placebo, affects GI symptoms assessed by questionnaires and regional GI transit times using the 3D-Transit system (paper IV).

Key results:

- Gastrointestinal symptoms were present during oxycodone treatment compared to placebo treatment including constipation, straining or squeezing to pass stool, incomplete bowel movement, hard stools, abdominal pain, and bloating (all $P<0.05$).
- Total GI transit and colorectal transit was significantly prolonged during oxycodone treatment ($P<0.005$) compared to placebo treatment. Within the colorectal segment, a *post hoc* analysis revealed increased transit time in the caecum/ascending colon ($P=0.01$) and the rectosigmoid colon ($P=0.04$).

Interpretation: Experimental OIBD was evoked during oxycodone treatment according to questionnaire scores. Furthermore, objective information on transit times revealed the most pronounced delays occur in the caecum/ascending colon and the rectosigmoid colon. This model has great potential for future interventional studies to discriminate efficacies of different laxative and opioid treatments.

4.5 Aim V

The results from the sphincter function assessments have been investigated preliminarily but due to the complexity of these data a more elaborate data analysis is warranted. Nonetheless, the preliminary analysis reveals large variability, and no evidence of oxycodone-induced alterations in sphincter function and distensibility.

Chapter 5 Discussion

In the present thesis a new experimentally induced model of OIBD in healthy volunteers was evaluated. The efficacy of the dosing regimen was validated through the development of analgesia to painful stimuli with muscle pressure algometry to the dorsal forearm. Furthermore, the induction of the most prevalent OIBD symptoms was determined by significant increases in all subjective bowel function questionnaires. Finally, objective quantification of four key aspects of OIBD was assessed with four novel methods and two of these detected clear changes in bowel function brought on by oxycodone treatment: MRI-based assessment of segmental colorectal volumes detected a clear increase in the proximal colon segments, while the 3D-Transit system found prolonged total GI transit time and within the colon; prolonged transit time in the caecum/ascending colon and rectosigmoid colon. On the other hand, no changes were detected in the electrogenic epithelial ion transport in isolated mucosal biopsies from the sigmoid colon and it has yet to be determined whether sphincter function and distensibility at rest and during challenge testing was affected by oxycodone treatment.

The first part of this discussion contains methodological considerations regarding the use of MRI, Ussing chambers, and 3D-Transit to assess bowel function. The second part of the discussion revolves around the validity of the model itself and the feasibility of quantifying OIBD objectively with these modalities.

5.1 Methodological considerations

5.1.1 MRI

Data obtained with MRI was used to compute segmental colorectal volumes at different time points. The scans provide a snapshot of the current colorectal filling, but do not provide any temporal information on for example GI motility. Because evaluation of colorectal volume with the Colometry segmentation software is based on manually placed regions of interests, this introduces an observer bias. To investigate this issue we have previously conducted an inter-observer study that showed negligible inter-observer bias in 14 MRI scans from four different HVs (Sandberg et al. 2015). Still, the colon is a highly dynamic organ and it is reasonable to expect large inter-individual and intra-individual variation, e.g. as a consequence of variety in diet and exercise levels. Nevertheless, the results from study I indicate that the inter-individual variability is sufficiently low to gain meaningful information with this method, although a certain degree of standardisation is required. The results from Pritchard and colleagues who previously published a paper on the normal ranges of colorectal volumes in 75 HVs, support our findings (Pritchard et al. 2014). Still, it should be kept in mind that the homogenous population of healthy males in our study is not representative of the general population and in particular not of the often heterogeneous patient groups. Rather, the model should be viewed as a characterisation of gut physiology in a controlled environment to be used as a model in e.g. interventional studies exploring the efficacy of different drugs on OIBD. The feasibility is supported by

the semi-automatic method that reduces time expenditure and greatly improves applicability of the model and reduces the cost. The present application provides a quantitative snapshot with high spatial resolution of the colorectal volume distribution that is far superior to the quantification of constipation with standard abdominal x-ray, but it does not provide any temporal information in terms of e.g. gut motility or transit times. An increase in spatial resolution as well as information on transit can be gained by performing a series of contiguous scans at fixed time intervals or by combining the method with radio-opaque markers.

5.1.2 Ussing chamber

The current literature on gut secretion and opioids are mainly focused on Ussing chamber studies where chemical compounds with opioidergic agonistic/antagonistic properties are applied directly to mucosa specimens and no studies currently exist where the opioid exposure occurs *in vivo*. Accordingly, literature has been sparse to guide the present experiments. Nevertheless, the Ussing chamber was chosen due to its widespread applications and its previously proven aptitude in detecting opioid-mediated effects on gut secretion albeit in different settings (Kachur et al. 1980; Kromer 1995; Sun et al. 2011; Fei et al. 2010; Poonyachoti & Brown 1999; Poonyachoti & Brown 2001). The majority of Ussing studies in human tissue rely on large tissue specimens from e.g. surgical resections (Brzuszczak et al. 1996; Archampong et al. 1972; Van De Kerkhof et al. 2006; Collins et al. 2011), which obviously is an option only during the presence of pathophysiological conditions such as colorectal carcinomas. For obvious reasons, a less invasive approach in HVs was required, which is the reason for the use of rectosigmoid mucosa biopsies. The method performed satisfactorily as the biopsies were both viable and responded to the added compounds as described in previous literature. However, intervention with oxycodone vs. placebo treatment did not show any appreciable difference. The reason is possibly that the biopsies were too superficial to include the enteric nervous system and that any effect on the enteric nervous system caused by opioid treatment was not present in the isolated mucosa. Assessment of opioid binding directly to the opioid receptors in the enteric nervous system, representing a “pure neurogenic effect” would obviously have been preferable, but this was not possible because of the risk of perforation during biopsy extraction. It can be argued that the electrogenic properties of only the mucosa do not reflect whether or not opioid treatment altered the colonic secretion.

Evidence of opioid receptors being expressed on immune cells in the intestinal mucosa have been presented previously (Sternini et al. 2004). If in fact the rectosigmoid mucosal biopsies had contained such cells, we would have expected that the application of morphine in the Ussing chamber to bind to their opioid receptors and consequently alter the electrogenic properties. As this effect was not demonstrated, we do not believe that opioid receptor-presenting immune cells were present in the rectosigmoid mucosa.

Another approach to obtain a quantifiable measure of altered gut secretion could be to measure stool water content using a freeze drying procedure (Hebden et al. 1999). Because stool water

content is also dependent on food and fluid intake, which varies greatly between individuals, this method would benefit from simultaneous collection of urine and either complete standardisation of food and fluid intake or more feasibly, precise monitoring of individual food and fluid intake. Other methods such as stool osmolality or electrolyte assays are conventionally used to determine the aetiology of diarrhoea (e.g. factitious diarrhoea, cholera toxin-induced diarrhoea (Steffer et al. 2012), and congenital chlorridorrhoea (Caspary 1986)) and may be of limited use in a constipation context as this method normally requires loose, watery faeces.

5.1.3 3D-Transit system

The 3D-Transit system provides a safe and minimally invasive ambulatory assessment of GI transit. The system was tolerated well by all subjects with only minor complaints on the system being uncomfortable during sleep or being slightly hot during the summertime. Capsule systems generally provide non-stationary recordings of gut motility and information on transit patterns rather than on site-specific motility patterns is provided. The 3D-Transit system has previously been validated against transit times obtained with radio-opaque markers with which it correlated well (Haase et al. 2014). However, previous studies investigating the effect of opioids on GI motility and transit times have typically relied on scintigraphy and breath tests, which generally measure gastric emptying and small intestinal transit (Maurer et al. 1996; Yee et al. 1991; Thorén et al. 1989; Kaufman et al. 1988; Jeong et al. 2012; Prokop et al. 1988)). The ability of the 3D-Transit system to obtain whole gut and regional transit data under normal physiological conditions with or without an intervention is advantageous compared to these methods. Furthermore, because the 3D-Transit data is based on exact anatomical localisation accompanied by site-specific contraction frequencies it holds great potential because it enables study of not only transit times but also more detailed information of gut motility and contraction patterns (i.e. non-propulsive contractions vs. propulsive contractions and retrograde movement vs. anterograde movements). However, the interpretation of this dimension is complicated by movement artefacts introduced by postural changes and movement of the 3D-Transit detector. The detector has a built-in accelerometer that eventually will enable removal of these artefacts but for the time being further software development is required.

Another limitation of this method in the present study design is the ingestion time of the 3D-transit capsule. The capsule was ingested immediately after the first dose of oral oxycodone along with a standardised meal. Because oral prolonged-release oxycodone is effective within 45 minutes with a peak plasma concentration within three hours and a half-life of approximately 4.5 hours it is highly unlikely that the oxycodone had time to take effect before the capsule was ingested. Therefore, it is not surprising that an oxycodone-induced difference in gastric emptying and small intestinal transit time was not found. On average, the capsule had traversed both stomach and small intestine within 8 to 9 hours, at which point the oxycodone treatment may not yet have taken adequate effect in the ENS. For future application the window between first dose and capsule ingestion should be prolonged.

Concerning capsule expulsion, an interesting discovery was made by Wang et al. who investigated regional and whole gut transit times in 215 HVs (Wang et al. 2015). Here they found that the wireless motility capsule was often expelled with the first bowel movement of the day. This means that data for whole gut transit time is not normally distributed; rather it is clustered at values separated by 24 hours. The same is likely true for the 3D-Transit capsule. However, because we measured altered GI function rather than normal GI function results may not be directly comparable. Not only did we change GI motility through oxycodone treatment, the ability of opioid treatment to decrease sensitivity to 'fullness' of the rectum which decreases the urge to defecate has previously been demonstrated (Musial et al. 1992). Therefore, we would not expect a normal bowel 'rhythmicity' in opioid-treated HVs. Furthermore, the main advantage of the 3D-Transit system compared to the wireless motility capsule is its capacity to determine the exact location in the GI tract of the capsule. This clustering of data in 24-hour intervals is associated only with capsule expulsion, meaning that when the capsule arrives in the rectum it will often be stored here until the next morning defecation. Again, this means that the discovery that transit is prolonged in the caecum/ascending colon will not have been affected by this phenomenon while the prolonged transit of the rectosigmoid colon may have been.

5.2 Experimental OIBD model in healthy volunteers

Regarding the model itself, clinical OIBD has been described in detail in the current literature, but to the best of the authors' knowledge no experimental model in healthy volunteers currently exist (Pappagallo 2001; Brock et al. 2012). The applied oxycodone dose in the present study was given great consideration before initiating the study. On one hand it was imperative that the dose regimen was sufficiently high to produce as pronounced level of GI adverse effects as possible. On the other hand, and more importantly, there were ethical and safety concerns that required the dose regimen to be sufficiently low to not pose a risk for the HVs in terms of overdose, development of substance dependence, or withdrawal of consent because of intolerable adverse effects (expectedly those of the CNS such as sedation, dizziness, nausea). When the dose regimen was ultimately settled on 5 mg twice daily on day 1, 10 mg twice daily on day 2-4, and 10 mg once daily on day 5 it was based on previous experience with similar daily doses of opioids in HVs (Staahl et al. 2006; Olesen et al. 2010; Staahl et al. 2011; Brokjaer et al. 2015) and because this is a clinically relevant dose (albeit a rather low dose when discussing chronic pain disorders). Furthermore, when comparing the dose regimen with those of chronic pain, this experimental setup was also extremely short-term with only five days of treatment. Still, the present study population were healthy and opioid naïve so a cautionary approach was preferable. Despite these contrasts between the present experimental model of OIBD and true clinical OIBD, effects of oxycodone treatment was clearly demonstrated not only with subjective questionnaires, but supported by objective quantification with novel methods. The relevance of the applied dose regimen was substantiated with the demonstration of analgesia during pressure algometry. All of these factors combined are what makes up the 'novel approach to assess OIBD'; a successful model in healthy volunteers that is highly unique and although the present study

population was highly homogenous it is feasible that the findings can be translated from 'bench to bedside' because the clinical manifestations of OIBD occur irrespective of age, gender, ethnicity.

The model was capable of detecting oxycodone-induced alterations in both subjective and objective measures. However, no clear correlations between subjectively perceived symptoms and objective quantification of OIBD could be determined which is not unusual. For example, agreement between the patients' own reports of GI function and objectively measured colonic transit time is modest (Chaussade et al. 1989; Ashraf et al. 1996). On the radiological side, assessments of colonic faecal loading with plain radiography and radio-opaque markers does not correlate with the subjective sensation of constipation (Grotz et al. 1994; Cowlam et al. 2008). Even simple measures such as stool frequency and consistency may not be valid, as many opioid-treated patients report normal stool frequency, but experience symptoms of OIBD such as straining, bloating, and the feeling of incomplete bowel evacuation (Bell et al. 2009). Therefore, measures based on objective clinical assessment may not be valid for capturing the patient experience. Furthermore, there are several methodological parameters that could add to the absent correlations: First, as demonstrated with the PAC-SYM questionnaire in paper II, the onset of OIBD symptoms are gradual – on day 2 no difference in symptom score occurs, but on day 3 through day 5 the symptoms gradually increase and would likely have increased even further if treatment had not been stopped on day 5. This provides a partial explanation for the absent correlations between subjective and objective scores: OIBD gradually manifests and it is likely that subjective and objective scores will start to correlate with prolonged opioid exposure. As this is not feasible with healthy volunteers, the next obvious step is to apply these methods in actual chronic pain patients, who are in long-term opioid therapy, although the comorbidity, concomitant medication, reduced mobility and other confounding factors are introduced with these subjects. Alternatively, it might be possible to prolong opioid exposure in HVs with reductions in dose regimen and close monitoring of physical/psychological well-being. Another explanation for the absent correlations could be that while the objective measures are very well defined (e.g. volume and transit time), the subjectively perceived symptoms encompass a plethora of clinical manifestations (e.g. bloating, constipation, abdominal pain, xerostomia, etc.) and it varies greatly on an individual basis which symptom(s) will dominate the consciousness. Such heterogeneous clinical presentation will obviously counteract the detection of correlations but may become more uniform with prolonged exposure. Regarding the safety of the model, no alterations to subjects' anxiety level occurred as a result of oxycodone treatment (paper IV). Similarly, no subjects showed evidence of substance dependence after oxycodone treatment had ceased (paper III). Therefore, this experimental model of OIBD appears to be well-tolerated and safe, which ensures its future application in investigating the underlying pathophysiology of OIBD in greater detail in a controlled environment free from the clinical confounders. Another possible application would be in studies investigating the efficacy of different opioids and laxative strategies, or the off-label efficacy of e.g. selective serotonin reuptake inhibitors to improve gut motility (Janssen et al. 2010). Currently,

assessment of laxative efficacy is based on SBMs and questionnaire-based assessments. Although these tools have proven efficient in the present study, we believe objective assessment provides important complementary information on the underlying mechanisms.

5.2.1 MRI

The objective MRI-based volumetric technique adds an interesting layer of information on top of the subjective questionnaire data. During oxycodone treatment a volume increase was found in the most oral colorectal segments (i.e. caecum/ascending colon and the transverse colon) while volume in the more anal segments remained relatively stable. Recent advances in detailing colonic motor patterns with high-resolution manometry may be key in understanding this proximal colonic volume increase during oxycodone treatment (Dinning et al. 2014). The same technique has been used to show that the normal increase in propagating motor patterns including high amplitude propagating sequences (mass movements) that occur after a meal is absent in patients with slow transit constipation (Dinning et al. 2015). The fact that opioids decrease gut motility coupled with the finding that high amplitude propagating sequences consistently originate in the proximal colon may provide a possible explanation for the volume accumulation we detect in the caecum/ascending colon and transverse colon if the high-amplitude propagating sequences are weakened due to treatment with oxycodone (Dinning et al. 2014). Conversely, the rectosigmoid volume remains relatively stable, which likely indicates that subjects were not experiencing opioid-induced evacuation disorders.

A volume increase in the caecum/ascending colon volume was found during placebo treatment although this only accounted for a third of the difference observed during oxycodone treatment. In paper I the inter- and intra-individual variability of the caecum/ascending colon displayed the most pronounced level of variability between observations, which may provide a partial explanation (Nilsson et al. 2015). Another explanation could be the presence of a systematic bias in the study design, which causes an increase in caecum/ascending colon volume on day 5. Subjects were scanned at the same time of day but were only in a fasting state on day 1. As deftly shown by Pritchard and colleagues, an acute increase in ascending colon volume of 10-20% occurs postprandially, which tallies with the observed increase during placebo treatment of 14% and provides a possible explanation for this volume increase (Pritchard et al. 2014). However, because the magnitude of volume increase observed during oxycodone treatment far surpasses that of the placebo treatment and of the postprandial data, we consider that most of the volume increase is due to the local binding of oxycodone in the ENS.

5.2.2 Ussing chamber

It has previously been shown that the modified Ussing air-suction chamber allows assessing epithelial ion secretion of small tissue samples, which enables studies of healthy human volunteers (Larsen et al. 2001; Osbak et al. 2007). Biopsies remain viable for hours following extraction, and thus the

modified Ussing air-suction chamber method was chosen to assess basic and stimulated ion transport, to explore the underlying *in vivo* pathophysiological mechanisms of experimentally induced OIBD in healthy human volunteers. Therefore, a systemic five-day oxycodone exposure was expected to target opioid receptors within the enteric nervous system and cause a neurogenic decrease in secretion in the gastrointestinal tract. Furthermore, it was hypothesised that this neurogenic mediated effect on gut secretion would potentially induce long-term alterations in e.g. activation of epithelial chloride channels that would be present locally in the isolated mucosa biopsies. Conventional opioids (e.g. morphine and oxycodone) elicit high affinity towards the ORs. However, as MORs are not located in human gut mucosa but is found in the submucosal and myenteric ganglia and nerve fibres in the myenteric plexus (De Luca et al. 1996; Sternini et al. 2004), our biopsies may have been too superficial to include the ENS, and therefore were insufficient to assess the tissue effect following oxycodone treatment. As no observable changes in SCC was shown in response to five-day oxycodone treatment when compared to baseline, several methodological considerations should be addressed, in order to verify these negative findings.

5.2.3 3D-Transit system

The 3D-Transit system has not been approved for MRI and therefore capsule retention on day 5 excluded MRI assessment. This caused the loss of MRI data in six subjects on day 5 during oxycodone treatment and in one subject on day 5 during placebo treatment. In itself, this highlights how effectively oxycodone treatment impacted normal gut function, but importantly, the six subjects that retained the 3D-Transit capsule on day 5 were *per se* also the six subjects with the longest transit time. Reasonably, these subjects were likely to also exhibit the most pronounced increase in colorectal volumes, which means that the MRI data are likely systematically underestimating the true increase in colorectal volumes following oxycodone treatment. Still, it is encouraging that the MRI and 3D-Transit findings compliment each despite this underestimation; both methods detect an impact of oxycodone treatment in the caecum/ascending colon. However, the 3D-Transit system also detects prolonged transit time in the rectosigmoid colon whereas no difference is found in volume. This might be due to the unaltered passive absorption of the colon during opioid treatment; although faecal matter is accumulating in the rectosigmoid colon the water content is continuously being passively absorbed and hence, the volume remains relatively stable despite increasing density of the faecal matter. This hypothesis is substantiated by the increasing scores of drier and harder stools observed with the BSFS questionnaire (paper II).

Chapter 6 Conclusions and Future Studies

In the presented new experimental model of OIBD, five-day treatment with oxycodone, compared to placebo, produces detectable alterations in GI function in healthy, opioid naïve males in terms of both subjective and objective aspects of gastrointestinal functions. No subjects withdrew their consent and no subject showed any evidence of increased anxiety or substance dependence as a result of the oxycodone treatment, which means that the study design is safe and well-tolerated.

Assessments of MRI-based segmental colorectal volumes with the novel Colometry segmentation software exhibit low variability and the method is sensitive both to changes in volume brought on by defecation and changes resulting from oxycodone treatment. The opioid-induced increase in volume colonic volume corresponds to the observed delay in colonic transit time detected with the 3D-Transit system. On the other hand, gut secretion in isolated mucosa from the rectosigmoid colon does not appear to be altered due to opioid treatment. Also, the anal sphincter function and distensibility appear to be highly variable and is awaiting a more exhaustive data analysis.

Ultimatel,y we successfully present a novel approach to assess OIBD in a controlled environment.

With an experimental model of OIBD in HVs there are numerous evident targets for future studies. For example, with this model it is possible to assess the efficacy of e.g. naloxegol, tapentadol, or targin vs. conventional laxative treatment in the alleviation of OIBD. In fact, the next sub-study of the MULTIPAIN6-2013 protocol (sub-study 2a) is focused on comparing the effect of 1) oxycodone prolonged release in combination with naloxone prolonged release with 2) oxycodone and macrogol treatment in a double-blinded, randomized trial in 20 healthy volunteers. This sub-study utilises the same methodology as sub-study 1b, although the Ussing chamber studies have been omitted and the FLIP method refined in order to look specifically at the rectoanal inhibitory reflex.

With this approach, the efficacy of different treatment strategies in terms of OIBD can be measured with not only self-assessed questionnaires and monitoring of SBMs but with objective, quantifiable measures as well. Using HVs in a controlled environment it is possible to gain insights into the pharmacological effect of study medication unmodified by comorbidity, concomitant pharmaceutical use, or pain-related psychosocial factors. Conclusions from such controlled settings are not necessarily representative of the actual patient population but the information will be no less important.

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Appendix: Paper I-IV

I. Nilsson M, Sandberg TH, Poulsen JL, Gram M, Frøkjær JB, Østergaard LR, Krogh K, Brock C, Drewes AM. Quantification and Variability of Colonic Volume with a Novel Magnetic Resonance Imaging Method. Neurogastroenterol Motil 2015 (in press)

II. Nilsson M, Poulsen JL, Brock C, Sandberg TH, Gram M, Frøkjær JB, Krogh K, Drewes AM. Opioid-induced Bowel Dysfunction in Healthy Volunteers Assessed with Questionnaires and Magnetic Resonance Imaging. Submitted: Eur J Gastroenterol Hepatol 2015.

III. Nilsson M, Brock C, Poulsen JL, Bindslev N, Hansen MB, Christrup LL, Drewes AM. Short-Term Oxycodone Treatment does not Affect Electrogenic Ion Transport in Isolated Mucosa from the Human Rectosigmoid Colon. Submitted: Scand J Gastroenterol 2015

IV. Poulsen JL, Nilsson M, Brock C, Sandberg TH, Krogh K, Drewes AM. The Impact of Opioid Treatment on Regional Gastrointestinal Transit. Submitted: J Neurogastroenterol Motil 2015.

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